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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

COMMENTARY

Therapeutic Trials With VP-16-213 and VM-26: Active Agents in Small Cell Lung Cancer, Non-Hodgkin's Lymphomas, and Other Malignancies [1]

Peter A. Radice,* Paul A. Bunn, Jr, and Daniel C. Ihde [2]

Since the late 1940s, podophyllotoxin, the crystalline extract of the plant *Podophyllum peltatum*, has been studied for its antitumor activity. Two synthetic congeners of podophyllotoxin, VM-26 and VP-16-213, have proven antitumor activity in animal studies and initial phase I and II trials. The chemical structures of the two epipodophyllotoxins are quite similar. Both have low molecular weights, are poorly soluble in water, and have limited stability in solution, therefore requiring complex formulations for parenteral administration. Podophyllotoxin binds to microtubules, inhibits their polymerization, and causes metaphase arrest of the cell cycle. VM-26 and VP-16-213 also cause an initial metaphase arrest without documented alteration of microtubular polymerization, followed by irreversible inhibition of cells entering mitosis (1–3). Both drugs also inhibit tritiated thymidine incorporation in fibroblast culture systems. Despite the similarities in chemical composition and mode of action of these compounds, there are differences observed in their activity against implanted tumors in animal models. Data on this activity in animal systems and early phase I and II clinical trials are well-summarized in the review by Rozencweig et al (4). Since that review was published, however, new oral preparations of VP-16-213 have been introduced and, with expanding clinical trials, the importance of the epipodophyllotoxins in the treatment of selected tumors has emerged. This commentary will highlight relevant clinical information from the review of Rozencweig et al, emphasize the data from new trials, and, where sufficient data exist, characterize the responsiveness of various tumors to the epipodophyllotoxins. We will also review the European literature on the pharmacokinetics and clinical efficacy of oral preparations of VP-16-213.

[1]Received Jan 22, 1979; revised Mar 28, 1979; accepted Apr 6, 1979.

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PHARMACOKINETICS

The pharmacokinetic properties of these drugs after iv infusion have principally been studied by Creaven and Allen (5–7). VM-26, after a single infusion of 67–90 mg/m^2, shows a triexponential decay curve. The half-life of the terminal phase varied from 11 to 38 hours. At 72 hours, 44% of the drug was recovered in the urine with 78% as metabolites. On the other hand, VP-16-213, after a single infusion of 220–290 mg/m^2, shows a biexponential decay curve, with a terminal half-life of 11.5 hours. After 72 hours, 44% of the drug was excreted in the urine with only 33% as metabolites. The three-times-larger plasma clearance and nearly six-times-larger renal clearance of VP-16-213 account for the three-times-larger equitoxic weekly doses of VP-16-213 (290 mg/m^2) versus VM-26 (90 mg/m^2). Despite animal and initial phase I human studies showing activity of VM-26 against brain tumors, cerebrospinal fluid levels of both compounds are $< 1\%$ of simultaneous plasma concentrations.

There are fewer published series on the pharmacokinetics of orally administered VP-16-213. In an early phase I trial with the oral lipophil capsule, Nissen et al (8) found no dose-dependent hematologic toxic effects over a dose range of 120–300 mg/m^2. Subsequent studies with ^{3}H-VP-16-213 showed erratic absorption of this formulation.[3] However, two newer formulations, the "drinking ampule" and a hydrophilic soft gelatin capsule, appear to give consistent gastrointestinal absorption of 47%–50% in ^{3}H-labeling studies. These oral formulations exhibited dose-dependent toxic effects in phase I studies and have less gastrointestinal toxicity than the initial capsule (9–11). Nissen et al (9) showed that the hematologic toxicity of the "drinking ampule" was similar to that of the iv formulation, but the low response rate in this study did not allow them to demonstrate equipotent antitumor activity of the oral compound. Although only one study has compared the oral versus the iv route for tumor response (12), preliminary cumulative data in small cell carcinoma of the lung (SCCL) suggest equipotent antitumor activity (vide infra).

[3]Sandoz Laboratories. Personal communication.

TABLE 1.—Antitumor activity of VP-16-213 in lung cancer

Cell type	Ref Nos.	No. of patients	No. of responses		Objective response rate (%)	Median duration of response (Days)
			Complete response	Partial response		
Small cell	8–19	201	14	74	44	56–225
Large cell	8–13	11	0	1	9	—
Squamous cell	8–13,20	30	0	5	17	90,133,181,193,218
Adenocarcinoma	8–13,20	31	0	3	10	61,105,135

ANTITUMOR ACTIVITY

Lung Cancer

The highest documented response rates of the epipodophyllotoxins are in SCCL. Cumulative figures from both phase I and II trials of VP-16-213, using differing doses and schedules, show an objective response rate (complete and partial response) of 44% (table 1). These studies include patients treated with less than optimal dosage schedules in the early phase I investigations. Moreover, the majority of the phase I and II trials include patients previously treated with a variety of chemotherapeutic agents. Despite these facts, there were 14 (7%) complete responses and 74 (37%) partial responses, for an objective response rate of 44% in these 201 patients. Thus, VP-16-213 is one of the most active single agents in SCCL.

Data reported from different institutions are remarkably similar, with objective response rates in phase II trials varying from 25% to 50% (13–20). The most important factors determining the response rates appear to be the amount of prior treatment and the dose and schedule used. In 109 previously treated patients, response rates varied from 20% to 48%, with an average response rate of 33%. Median durations of remission of from 92 to 110 days are reported. On the other hand, 90 untreated patients had an average response rate of 50%, with median durations of remission of from 50 to 225 days (average, 180).

The response to VP-16-213 in SCCL also appears to be dose- and schedule-dependent (table 2). Initial trials in animal tumor models showed that smaller, more frequent doses of the epipodophyllotoxins gave greater increases in lifespan than large, weekly doses (1). Therapy with doses of 60 mg/m^2 iv qd × 5 every 2–4 weeks or 125–140 mg/m^2 iv qod × 3 every 2–4 weeks appears to be superior to weekly administration of 200–300 mg/m^2 in human trials as well (table 2). However, most of the patients given a weekly schedule had been previously treated (17,18). Oral administration appears to give equivalent, if not superior response rates when compared to iv administration. In a randomized trial comparing oral doses of 300 mg/m^2 qd × 5 every 3 weeks and 160 mg/m^2 qd × 3 every week with iv doses of 300 mg/m^2 weekly, Cavalli et al (18) found response rates of 64%, 42%, and 20% respectively. However, patients receiving the oral doses had slightly more hematologic toxicity than those receiving the iv drug, raising the possibility that the two forms of drug administration were not equitoxic.

At least ten phase III trials are in progress to test VP-16-213 in combination with other active agents (21,22).[4]

TABLE 2.—Schedule-dependent responses to VP-16-213 in SCCL

Schedule	Ref Nos.	Dose (mg/m^2)	No. of patients	Previous treatment (%)	No. of responses		Objective response rate (%)
					Complete response	Partial response	
Iv administration							
5 days/wk q2–4wks	11,13,14,19	60	59	58	9	19	47
qod × 3 doses q2–4wks	15	125–140	13	0	0	7	54
Weekly	17,18	200–300	36	68	1	7	22
Total			108		10	33	40
Oral administration							
5 days/wk q2–4wks	8–12,16,18	60–300	76	68	2	32	45
3 days/wk weekly	18	166	17	18	2	9	64
Total			93		4	41	48

TABLE 3.—Antitumor activity of epipodophyllotoxins in lymphoma/leukemia

Tumor	VM-26					VP-16-213				
	Ref Nos.	No. of patients	No. of responses: Complete response	No. of responses: Partial response	Objective response rate (%)	Ref Nos.	No. of patients	No. of responses: Complete response	No. of responses: Partial response	Objective response rate (%)
Non-Hodgkin's lymphomas										
Reticulum cell sarcoma/diffuse histiocytic lymphoma	24–30	71	0	30	42	8,9,11,28,33–44	111	1	35	32
Lymphosarcoma	24,27–30	48	0	22	45	33	22	0	5	23
Unspecified	—	—	—	—	—	40	52	0	14	27
Hodgkin's disease	24–30	92	3	30	36	10,28,33,34,36,40	64	0	9	14
Acute nonlymphocytic leukemia	26,31	14	0	1	7	8,10,13,28,30,31, 36,37	158	19	19	24
Acute myelomonocytic leukemia	—	—	—	—	—	28,30,36,37	34	10	4	41
Acute lymphocytic leukemia	31,32	29	1	3	14	11,28,30,31	44	1	2	7

Preliminary data in abstract form have been published for five of these trials.[4] While the complete response rate of combined therapy trials appears to be higher than that of the single-agent trials, as would be expected, it is too early to make conclusive statements.

As shown in table 1, there is much less experience with VP-16-213 administration in non-SCCL. The response rate of 17% in squamous cell carcinoma is similar to the results obtained with CCNU and cyclophosphamide and suggests that VP-16-213 is a potentially active drug worthy of evaluation in phase II and III studies. Although only a 10% response rate is reported in large cell carcinoma and adenocarcinoma, similar to response rates with most other single agents, only 42 patients have been treated.

VM-26 has not been adequately tested in lung cancer. There has been one response among nine patients with SCCL treated with varying schedules (23,24).[5] In non-SCCL, there have been no responses among 28 patients.[5]

Lymphoma/Leukemia

Although the number of evaluable patients in each histologic category is adequate, many patients were treated within broad phase I and II studies in which the definitions of response, the durations of remission, and sufficient information to determine if adequate dosage schedules were employed are not provided. In many of the European trials the Rappaport classification of the non-Hodgkin's lymphomas was not used, making comparisons with the results of other investigators even more difficult.

When the data on reticulum cell sarcoma and diffuse histiocytic lymphoma are combined, the objective response rates for VM-26 and VP-16-213 are 42% and 32% respectively (table 3). Moreover, 94% of the evaluable patients had had previous chemotherapy. In the National Cancer Institute (Bethesda, Md) trial, of 13 patients with diffuse histiocytic, mixed, or undifferentiated lymphoma who had previous extensive combination chemotherapy, one complete remission and five partial remissions were seen, with a median duration of remission of 5.5 months (40). In the only untreated patients reported in the literature, Jacobs et al (35) observed responses in three of five patients. Thus, VP-16-213 is as active as cyclophosphamide, vincristine, Adriamycin, bleomycin, or BCNU in diffuse histiocytic lymphoma, since response rates with the latter agents of 25%–58% in previously treated patients have been reported (41).

In lymphosarcoma, objective response rates of 45% and 23% are reported for VM-26 and VP-16-213 respectively (table 3). The number of evaluable patients with these histologic types is smaller. However, these response rates also compare favorably with those of cyclophosphamide, bleomycin, and Adriamycin (37%–44% in previously treated patients) (41).

All the evaluable patients with Hodgkin's disease included in the trials employing the epipodophyllotoxins had had prior combination chemotherapy with or without radiation therapy. VM-26 showed an objective response rate of 36% in Hodgkin's disease with three complete

[4]Annual Report to the Food and Drug Administration, VP-16-213. Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

[5]Annual Report to the Food and Drug Administration, VM-26. Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute.

responses among the total of 92 evaluable patients (table 3). Sonntag et al (27) reported a median remission duration of 128 days (range, 26–516+) in five responders. In the few other studies where remission duration is noted it is usually in the order of 1–2 months in those patients treated with prior combination chemotherapy. VP-16-213, on the other hand, had a response rate of 14% in Hodgkin's disease with no complete remissions. However, the number of VP-16-213-treated cases is small, with 17 patients receiving inadequate doses. These results are also similar to those obtained with other recently introduced single agents (eg, DTIC, 56%; bleomycin, 50%; BCNU, 47%; Adriamycin, 33% [42]) in similar heavily pretreated patients.

A limited number of phase III trials to evaluate combinations containing VM-26 have been initiated in patients with lymphoma. Eckhardt et al (43), using VM-26, procarbazine, and prednisolone in 35 treated and 15 untreated patients with Hodgkin's disease, observed 40% complete remissions with an 85% objective response rate. All responses occurred within three cycles. Misset et al (44), employing Adriamycin, VM-26, cyclophosphamide, and prednisone, obtained complete remissions in 58% of patients and partial responses in another 17% in non-Hodgkin's lymphoma patients. At present, there are at least five ongoing protocols studying combinations including the epipodophyllotoxins in the treatment of the malignant lymphomas (23,24).[6] With the proven efficacy of combination chemotherapy in the malignant lymphomas, there are no phase III single-agent trials in untreated patients comparing the epipodophyllotoxins to other agents or combinations. A randomized trial comparing a combination regimen, with or without the addition of an epipodophyllotoxin, would presumably be the most acceptable method to directly test their efficacy in untreated patients, but no such trials are presently underway.

The objective response rate of VP-16-213 is 25% in patients with acute myelogenous leukemia (AML), while the response rate is 41% in the subgroup of patients with acute myelomonocytic leukemia (AMMoL) (28,30,36). This apparent specificity for monocytic precursors has been reported from Mathé's group alone, while others have not found such striking results in AMMoL (37). VM-26 has not been adequately tested in AML.

Surprisingly, neither drug has shown significant activity against acute lymphocytic leukemia, with response rates of 14% and 7% for VM-26 and VP-16-213 respectively. However, Rivera et al (45) reported a 30% marrow response rate in 33 patients with late-stage, resistant disease in a phase III study combining VM-26 with cytosine arabinoside.

There have been several reports of "complete remissions" in mycosis fungoides with only seven evaluable patients (38,39). Further study is warranted in advanced mycosis fungoides patients to confirm these reports.

Other Tumors (Table 4)

VM-26 appeared to produce improvement in two patients with astrocytoma in an initial phase I study by Muggia et al (23). Earlier animal studies predicted moderate central nervous system activity with modest (36%–56%) increases in lifespan in mice injected with ten L1210 cells ic after ip VM-26 (1). Creaven and Allen (5), however, demonstrated that cerebrospinal fluid levels of VM-26 in three patients were 1% that of simultaneous plasma levels, with the highest level, 27%, in a patient following intracranial surgery and irradiation. With further investigation in man, the activity of VM-26 in intracranial tumors has been confirmed with a cumulative response rate of 52% in 23 patients (table 4). Many of these patients had had prior therapy with nitrosoureas. The response rates are difficult to interpret since they include patients with clinical improvement who had no measurable lesions. Sklansky et al (46) and Walker (47), using stricter criteria, reported five objective responses in 13 patients with measurable lesions, with a mean remission duration of 8 months. The seven patients who were asymptomatic stage I demonstrated a median time to progression of 15 months (46). These results appear favorable in comparison to median survivals of 6–7 months in patients treated with radiation therapy or surgery (47). These response rates and durations of response appear equivalent to results achieved with the best single agents including BCNU and procarbazine. BCNU, the best available nitrosourea, gives clinical responses in approximately 50% of patients, with a 6-month median survival. Procarbazine is reported to yield a 52% response rate with similar survivals. Most other agents, including cyclophosphamide, Adriamycin, 5-fluorouracil, and mithramycin, are all reported to have no activity (47). Rivera et al (48) have also reported responses in children with neuroblastoma. There were three objective responses among 14 patients, with nine other patients showing improvement. Early phase III studies combining VM-26 with the nitrosoureas or Adriamycin in intracranial neoplasms are underway.[7]

VP-16-213 has had inadequate trials in intracranial tumors, perhaps due to its lesser activity in animal models. However, in six reported cases there have been two symptomatic improvements, suggesting that further studies may be warranted.[8]

VM-26 has shown moderate activity in bladder cancer. There was an objective response rate of 32% among 142

[6] *See* footnotes 4 and 5.

[7] *See* footnote 5.

[8] *See* footnote 4.

TABLE 4.—Activity of epipodophyllotoxins in solid tumors

Tumor	Ref Nos.	VM-26				VP-16-213			
		No. of patients	No. of responses: Complete response	No. of responses: Partial response	Objective response rate (%)	No. of patients	No. of responses: Complete response	No. of responses: Partial response	Objective response rate (%)
Active (> 20% response in < 20 patients)									
Intracranial	46–50*	23	0	12	52	6	0	2	33
Ovarian	10,13,14,26,36,51,52*	20	1	7	40	40	0	4	10
Bladder	10,53–55	142	14	32	32	20	0	1	5
Testicular	13,24,26,33,56,57*	6	0	0	—	21	0	8	38
Inactive (< 20% response in > 20 patients)									
Colorectal	10,26,33,36,58–60	6	0	0	—	157	0	6	4
Breast	8–11,36,61,62†	22	0	2	9	138	1	6	4
Renal	36	5	0	0	—	109	0	3	3
Melanoma	9,10,33,34,63	4	0	0	—	76	0	1	1
Head and neck	33,34,36	16	0	0	—	35	0	0	—
Soft tissue sarcoma	8,9,31,36	20	0	0	—	34	—	2	6
Insufficient data									
Hepatoma	64	—	—	—	—	6	0	3	50
Gastric	9,26,33,36,55	6	0	0	—	19	0	2	10
Esophageal	26,33	15	0	0	—	9	0	2	22
Prostatic	13,14	—	—	—	—	4	0	0	—
Mesothelioma	9,10,33	—	—	—	—	7	0	0	—

*See footnote 4.
†See footnote 3.

evaluable patients with undefined stages (table 4). In T1–S lesions, iv VM-26 caused complete disappearance of papillomatous lesions in 32% of patients and partial responses in 22%, with significant differences in rates of recurrence when compared to a control group. Patients in this study with stage T3–4 disease showed a response rate of 24% (65). Intravesicular VM-26 has also been used with initial good results in early-stage patients and as adjuvant therapy in patients after resection (66). However, a European Organization for Research on Treatment of Cancer trial currently reveals no advantage for prophylactic intravesicular administration.[9] VP-16-213 has shown little response in bladder carcinoma.

In previously treated patients with advanced ovarian carcinoma, objective response rates for VM-26 and VP-16-213 were 40% and 10% respectively (26,52).[8] Most of these patients were treated in broad phase II trials with little staging data available. Jankowski and Vahrson (51), however, reported one complete remission and seven partial remissions in 19 patients with advanced ovarian cancer treated with VM-26. The response rate of 38% in 21 patients treated with VP-16-213 for testicular carcinoma is also impressive (13,24,33,56,57)[8]; however, all the patients were treated in phase I and broad phase II trials. Therefore, further specific studies will be required in both diseases to substantiate these initial findings.

VP-16-213 may have activity against hepatocellular carcinoma. In six patients treated only with VP-16-213 there were three partial responses, while in six more patients treated with VP-16-213 and 5-fluorouracil two responded (64). Responses of thyroid carcinoma to VP-16-213 have also been reported, mostly in the form of single case reports (67).

Neither drug has appreciable activity in breast, colorectal, renal, or head and neck carcinomas, melanoma, or soft tissue sarcoma. Insufficient patient numbers preclude drawing conclusions in gastric, esophageal, and prostate cancers, mesothelioma, or hepatoblastoma.

TOXIC EFFECTS

Table 5 shows toxicity data from those studies in which this information was presented. Inherent inaccuracies due to differing dosage schedules, prior therapy, and differing criteria for defining toxicity cannot be avoided.

The major dose-limiting toxicity for both VM-26 and VP-16-213 is hematologic. In 28% and 43% of the patients given VM-26 and VP-16-213 respectively, leukopenia was

[9]Rozencweig M. Personal communication.

TABLE 5.—Toxicity of epipodophyllotoxins§

	No. of patients with toxicity/ total No. treated with VM-26	%	No. of patients with toxicity/ total No. treated with VP-16-213	%
Hematologic				
Anemia	2/21	9.5	67/158	42
Leukopenia (cells/mm^3)	119/425	28	378/877	43
2000–3000	21/42		29/177	
1000–2000	11/43		42/181	
< 1000	26/128		19/189	
Thrombocytopenia (cells/mm^3)	47/296	16	109/807	14
50,000–100,000	11/43		27/171	
< 50,000	9/50		12/188	
Gastrointestinal	92/642	14	45/330*	15
Nausea	35/115		55/347	
Vomiting	5/64		19/97	
Diarrhea	7/97		1/33	
Anorexia	5/31		—	
Stomatitis	3/185		15/283	
Alopecia	52/192	27	221/623	35
Arrhythmia/palpitation	15/19	—	2/34	
Mild LFT† elevation	8/44		—	
Hypotension	8/124		—	
Phlebitis‡	28/40		—	
Fever	—		7/283	
Peripheral neuropathy	9/19		9/199	

*Data for iv route alone; gastrointestinal toxicity with oral VP-16-213, 88 of 160 patients (55%).
†LFT = liver function test.
‡Change in solvent dilution eliminated phlebitis.
§*From* refs 8–20, 22–24, 26–31, 33, 34, 36, 40, 46, 48, 52, 53, and 55.

reported. In those studies that reported wbc nadirs, 20% were < 1000/mm^3 with VM-26 as opposed to 10% for VP-16-213. Thrombocytopenia was seen in 16% of those patients treated with VM-26 and in 14% of those treated with VP-16-213. In general, a gradation of hematologic toxicity was noted, with the most severe degrees in patients with lymphoma and lesser degrees in patients with solid tumors and intracranial neoplasms, reflecting both the underlying disease and the aggressiveness of previous therapy. The time course of the hematologic toxicity varied somewhat with dosage schedule, underlying disease, and prior chemotherapy. Generally, with an optimal regimen, the wbc nadir was reached in 10–14 days with recovery by Days 16–21. No cumulative toxicity has been noted.

Gastrointestinal toxicity occurred in approximately 15% of the patients with iv administration of either drug. However, oral administration of VP-16-213 increased this toxicity to 55%. Stomatitis was also noted in 23% of the patients receiving oral compounds as opposed to only 5% receiving iv compounds. Oral administration does not markedly differ from the iv route in all other types of toxicity. Alopecia was seen commonly with both drugs. Fever, mild liver function test elevations, phlebitis, and palpitations were noted less frequently. Hypotension was rarely seen when the agents were given by iv infusions over 30–60 minutes and were never noted after oral administration of VP-16-213. Mild peripheral neuropathy was also noted in 10%–20% of the patients without relation to previous vincristine therapy. However, in combinations including both vincristine and the epipodophyllotoxins given simultaneously, serious additive toxicity has been seen.[10]

CONCLUSIONS

Rozencweig et al (4) extensively reviewed the experimental data of the mode of action of the epipodophyllotoxins and their animal antitumor activity and toxicology. From the early clinical trials that they presented, they could find no conclusive evidence suggesting a different spectrum of antitumor activity for these compounds. Al-

[10]Minna JD. Personal communication.

though their general conclusions remain valid, since their report appeared, more than twice the number of patients they reviewed have been treated with the epipodophyllotoxins in SCCL, lymphomas, and intracranial tumors.

At present, VP-16-213 appears to be as active as any single agent against SCCL, histiocytic lymphoma, and possibly other malignant lymphomas. While VM-26 may occupy a similar position with respect to intracranial malignancies, the limited phase I and II results will require confirmation in ongoing trials. Present phase III combination chemotherapy trials in these tumors will be of great interest, but unless there are specific studies comparing combinations with and without an epipodophyllotoxin, conclusive statements regarding the contribution of the epipodophyllotoxins will not be forthcoming. VM-26 also has proven activity in the treatment of neuroblastoma, Hodgkin's disease, and ovarian and bladder carcinomas. Further studies are required to confirm the efficacy of VP-16-213 in AML, thyroid carcinoma, and non-SCCL.

Optimal dosage and schedules for the epipodophyllotoxins are becoming clearer. Smaller intermittent doses given more frequently increased the lifespan in mice. With iv administration in human trials, weekly doses appear to give poorer results than daily doses for 3–5 days every 1–3 weeks.

European trials have noted the efficacy of new oral formulations which give less toxicity than the first oral compound available at the time of Rozencweig's review. Oral routes seem to have equipotent antitumor activity when compared to iv routes. Although the oral formulations have been made available by Sandoz Laboratories (Basle, Switzerland), there has been no attempt to obtain approval for clinical trials in the US. More studies are required to investigate the absorption, pharmacokinetics, and antitumor activity in comparison with optimal iv routes before widespread use of the oral route can be recommended. However, with the minimal risk of hypotension using the oral route and the convenience of this route for daily outpatient administration, oral VP-16-213 could become standard therapy in drug combinations for SCCL and other malignancies.

REFERENCES

1. Stahelin H. Activity of new glycosidic lignan derivative (VP-16-213) related to podophyllotoxin in experimental tumors. Eur J Cancer 9:215–221, 1973.
2. Stahelin H. 4′-Demethyl-epipodophyllotoxin thenylidene glucoside (VM 26), a podophyllum compound with a new mechanism of action. Eur J Cancer 6:303–311, 1970.
3. Loike JD, and Horwitz SB. Effects of podophyllotoxin and VP-16-213 on microtubule assembly in vitro and nucleoside transport in HeLa cells. Biochemistry 15:5443–5448, 1976.
4. Rozencweig M, Von Hoff DD, Henney JE, et al. VM 26 and VP 16-213: a comparative analysis. Cancer 40:334–342, 1977.
5. Creaven PJ, and Allen LM. EPEG, a new antineoplastic epipodophyllotoxin. Clin Pharmacol Ther 18:221–226, 1975.
6. Creaven PJ, and Allen LM. PTG, a new antineoplastic epipodophyllotoxin. Clin Pharmacol Ther 18:227–233, 1975.
7. Allen LM, and Creaven PJ. Comparison of the human pharmacokinetics of VM-26 and VP-16, two antineoplastic epipodophyllotoxin glucopyranoside derivatives. Eur J Cancer 11:697–707, 1975.
8. Nissen NI, Hansen HH, Pedersen H, et al. Clinical trial of the oral form of a new podophyllotoxin derivative, VP-16-213 (NSC-141540), in patients with advanced neoplastic disease. Cancer Chemother Rep 59:1027–1029, 1975.
9. Nissen NI, Dombernowsky P, Hansen HH, et al. Phase I clinical trial of an oral solution of VP-16-213. Cancer Treat Rep 60:943–945, 1976.
10. Falkson G, van Dyk JJ, van Eden EB, et al. A clinical trial of the oral form of 4′-demethyl-epipodophyllotoxin-β-D ethylidene glucoside (NSC 141540) VP 16-213. Cancer 35:1141–1144, 1975.
11. Lau ME, Hansen HH, Nissen NI, et al. Phase I trial of a new form of an oral administration of VP-16-213. Cancer Treat Rep 63:485–487, 1979.
12. Brunner KW, Sonntag RW, Ryssel HJ, et al. Comparison of the biologic activity of VP-16-213 given iv and orally in capsules or drink ampules. Cancer Treat Rep 60:1377–1379, 1976.
13. Jungi WF, Senn HJ, Beckmann C, et al. Therapeutic experiences using the new podophyllotoxin derivative VP 16-213 in malignant human tumors. Schweiz Med Wochenschr 105:1365–1369, 1975.
14. Jungi WF, and Senn HJ. Clinical study of the new podophyllotoxin derivative, 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213), in solid tumors in man. Cancer Chemother Rep 59:737–742, 1975.
15. Eagan RT, Carr DT, Frytak S, et al. VP-16-213 versus polychemotherapy in patients with advanced small cell lung cancer. Cancer Treat Rep 60:949–951, 1976.
16. Hansen M, Hirsch F, Dombernowsky P, et al. Treatment of small cell anaplastic carcinoma of the lung with the oral solution of VP-16-213 (NSC 141540, 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside). Cancer 40:633–637, 1977.
17. Cohen MH, Broder LE, Fossieck BE, et al. Phase II clinical trial of weekly administration of VP-16-213 in small cell bronchogenic carcinoma. Cancer Treat Rep 61:489–490, 1977.
18. Cavalli F, Sonntag RW, Jungi F, et al. VP-16-213 monotherapy for remission induction of small cell lung cancer: a randomized trial using three dosage schedules. Cancer Treat Rep 62:473–475, 1978.
19. Tucker RD, Ferguson A, Van Wyk C, et al. Chemotherapy of small cell carcinoma of the lung with VP 16-213. Cancer 41:1710–1714, 1978.
20. Eagan RT, Ingle JN, Creagan ET, et al. VP-16-213 chemotherapy for advanced squamous cell carcinoma and adenocarcinoma of the lung. Cancer Treat Rep 62:843–844, 1978.
21. Aisner J, Esterhay RJ, Jr, and Wiernik PH. Chemotherapy vs. chemoimmunotherapy for small cell carcinoma of the lung. Proc Am Assoc Cancer Res and ASCO 18:310, 1977.
22. Cohen MH, Ihde DC, Bunn PA, Jr, et al. Cyclic alternating combination chemotherapy for small cell bronchogenic carcinoma. Cancer Treat Rep 63:163–170, 1979.
23. Muggia FM, Selawry OS, and Hansen HH. Clinical studies with a new podophyllotoxin derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819). Cancer Chemother Rep 55:575–581, 1971.

24. European Organization for Research on Treatment of Cancer, Cooperative Group for Leukaemias and Haematosarcomas. Clinical screening of epipodophyllotoxin VM 26 in malignant lymphomas and solid tumours. Br Med J 2:744–748, 1972.
25. Trempe G, Sykes M, Young C, et al. Phase 1 trial of the podophyllotoxin derivative VM-26. Proc Am Assoc Cancer Res 11:79, 1970.
26. Dombernowsky P, Nissen NI, and Larsen V. Clinical investigation of a new podophyllum derivative, epipodophyllotoxin, 4′-demethyl-9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819), in patients with malignant lymphomas and solid tumors. Cancer Chemother Rep 56:71–82, 1972.
27. Sonntag RW, Senn HJ, Nagel G, et al. Experience with 4′-demethyl-epipodophyllotoxin 9-(4,6-*O*-2-thenylidene β-D-glucopyranoside), VM 26; NSC-122819, in the treatment of malignant lymphosis. Eur J Cancer 10:93–98, 1974.
28. Mathé G, Schwarzenberg L, Pouillart P, et al. Acute leukemia and various hematosarcomas. Trial treatment with 4-demethyl-epipodophyllotoxin beta D ethylidene glucoside VP 16213. Nouv Presse Med 3:521–524, 1974.
29. Mathé G, Schwarzenberg L, Pouillart P, et al.Therapeutic trials in various hematosarcomas with 4-demethylepipodophyllotoxin beta D thenylidene glucoside (VM 26 or EPT). Nouv Presse Med 3:447–451, 1974.
30. Mathé G, Schwarzenberg L, Pouillart P, et al. Two epipodophyllotoxin derivatives, VM 26 and VP 16213, in the treatment of leukemias, hematosarcomas, and lymphomas. Cancer 34:985–992, 1974.
31. Rivera G, Avery T, and Pratt C. 4′-Demethylepipodophyllotoxin, 9-(4,6-*O*-2-thenylidene-β-D-glucopyranoside) (NSC-122819; VM-26) and 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213) in childhood cancer: preliminary observations. Cancer Chemother Rep 59:743–749, 1975.
32. Rosenstock JG, and Donaldson MH. Phase I–II trial of VM-26 (NSC-122819) in the treatment of children with late-stage leukemia. Cancer Treat Rep 60:265–267, 1976.
33. Nissen NI, Larsen V, Pedersen H, et al. Phase I clinical trial of a new antitumor agent, 4′-demethylepipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 56:769–777, 1972.
34. Cecil JW, Quagliana JM, Coltman CA, et al. Evaluation of VP-16-213 in malignant lymphoma and melanoma. Cancer Treat Rep 62:801–803, 1978.
35. Jacobs P, King HS, and Sealy GRH. Epipodophyllotoxin (VP-16-213) in the treatment of diffuse histiocytic lymphoma. S Afr Med J 49:483–485, 1975.
36. European Organization for Research on the Treatment of Cancer, Clinical Screening Group. Epipodophyllotoxin VP16-213 in treatment of acute leukaemias, haematosarcomas and solid tumours. Br Med J 3:199–202, 1973.
37. Smith IE, Gerken ME, Clink HM, et al. VP 16-213 in acute myelogenous leukaemia. Postgrad Med J 52:66–70, 1976.
38. Jacobs P, King HS, and Gordon W. Epipodophyllotoxin in mycosis fungoides. Lancet 1:111–112, 1975.
39. Jacobs P, King HS, and Gordon W. Letter: Chemotherapy of mycosis fungoides. S Afr Med J 49:1286, 1975.
40. Bender RA, Anderson T, Fisher RT, et al. The activity of the epipodophyllotoxin VP16 in the treatment of combination chemotherapy resistant non-Hodgkin's lymphoma. Am J Hematol. In press.
41. Jones SE, Rosenberg SA, Kaplan HS, et al. Non-Hodgkin's lymphomas. Cancer 30:31–38, 1972.
42. DeVita VT, Lewis BJ, Rozencweig M, et al. The chemotherapy of Hodgkin's disease. Cancer 42:979–990, 1978.
43. Eckhardt S, Döbrentey E, and Bodrogi I. Results obtained with combination therapy of VM-26, Natulan and prednisolone in generalized Hodgkin's disease. Chemotherapy 21:248–254, 1975.
44. Misset JL, Pouillart P, Amiel JL, et al. Adriamycin, VM 26, cyclophosphamide and prednisone (AVmCP) combination in the therapy of disseminated lymphoreticulosarcomas. Nouv Presse Med 4:3117–3120, 1975.
45. Rivera G, Aur RJ, Verzosa M, et al. Treatment of refractory acute lymphocytic leukemia (ALL): combination chemotherapy with VM26 and cytosine arabinoside (ara-C). Proc Am Assoc Cancer Res and ASCO 18:285, 1977.
46. Sklansky BD, Mann-Kaplan RS, Reynolds AF, Jr, et al. 4′-Demethyl-epipodophyllotoxin-β-D-thenylidene-glucoside (PTG) in the treatment of malignant intracranial neoplasms. Cancer 33:460–467, 1974.
47. Walker MD. Chemotherapy: adjuvant to surgery and radiation therapy. Semin Oncol 2:69–72, 1975.
48. Rivera G, Green A, Hayes A, et al. Epipodophyllotoxin VM-26 in the treatment of childhood neuroblastoma. Cancer Treat Rep 61:1243–1248, 1977.
49. Gutin PH, and Walker MD. Iv methyl-CCNU, VM-26, and cranial irradiation in therapy for malignant brain tumors. Cancer Treat Rep 61:1715–1717, 1977.
50. Pouillart P, Mathé G, Thy TH, et al. Treatment of malignant gliomas in adults using a combination of Adriamycin, VM 26 and CCNU: results of a type II trial. Eur J Cancer 13:418–424, 1977.
51. Jankowski RP, and Vahrson H. The toxicity of massive doses of VM26 (4-demethyl-epipodophyllotoxin-β-D-thenylidene glucoside). A contribution to the therapy of advanced ovarian cancer. Med Klin 72:2122–2126, 1977.
52. Edmonson JH, Decker DG, Malkasian GD, et al. Phase II evaluation of VP-16-213 (NSC-141540) in patients with advanced ovarian carcinoma resistant to alkylating agents. Gynecol Oncol 6:7–9, 1978.
53. Pavone-Macaluso M, Caramia G, Rizzo FP, et al. Preliminary evaluation of VM-26: a new epipodophyllotoxin derivative, in the treatment of urogenital tumours. Eur Urol 1:53–56, 1975.
54. Pavone-Macaluso M, and European Organization for Research on the Treatment of Cancer Genitourinary Tract Cooperative Group A. Single drug chemotherapy of bladder cancer with Adriamycin, VM-26 or bleomycin. A phase II multicentric, cooperative study. Eur Urol 2:138, 1976.
55. Creaven PJ, Newman SJ, Selawry OS, et al. Phase I clinical trial of weekly administration of 4′-demethyl-epipodophyllotoxin 9-(4,6-*O*-ethylidene-β-D-glucopyranoside) (NSC-141540; VP-16-213). Cancer Chemother Rep 58:901–907, 1974.
56. Young CW, Ihde DC, and Von Stubbe W. Preliminary clinical trial of 4′demethylepipodophyllotoxin-β-D-ethylidene-glucoside (VP 16-213). Proc Am Assoc Cancer Res 14:60, 1973.
57. Newlands ES, and Bagshawe KD. Letter: Epipodophylin derivative (VP 16-213) in malignant teratomas and choriocarcinomas. Lancet 2(8028):87, 1977.
58. Douglass HO, Jr, MacIntyre JM, Evans JT, et al. Methyl CCNU (meCCNU) plus β-2′deoxythioguanosine vs VP 16 or diglycoaldehyde (DGA) in advanced, previously treated colorectal adenocarcinoma: a phase II study of the ECOG. Proc Am Assoc Cancer Res and ASCO 18:313, 1977.
59. Hahn RG, Bauer M, Walter J, et al. Phase II study of

single-agent therapy with megestrol acetate, VP–16–213, cyclophosphamide, and dianhydrogalactitol in advanced renal cell cancer. Cancer Treat Rep 63:513–515, 1979.

60. Perry MC, Moertel CG, Schutt AJ, et al. Phase II studies of dianhydrogalactitol and VP-16-213 in colorectal cancer. Cancer Treat Rep 60:1247–1250, 1976.
61. Ahmann DL, Bisel HF, Eagan RT, et al. Phase II evaluation of VP-16-213 (NSC-141540) and cytembena (NSC-104801) in patients with advanced breast cancer. Cancer Treat Rep 60:633–635, 1976.
62. Eagan RT, Ahmann DL, and Bisel HF. Evaluation of VP-16 and the combination of Adriamycin and vincristine in advanced breast cancer. Oncology 33:146–148, 1976.
63. Bellet RE, Catalano RB, Mastrangelo MJ, et al. Phase II trial of VM-26 in patients with metastatic malignant melanoma. Cancer Treat Rep 62:445–447, 1978.
64. Cavalli F, Tschopp L, Gerber A, et al. Therapeutic results using VP 16-213 alone or in combination with 5-fluorouracil in liver cancer (hepatoma). Schweiz Med Wochenschr 107:1960–1966, 1977.
65. Mechl Z, Rovný F, and Sopkova B. VM 26 (4-demethyl-epipodophyllotoxin-β-D-thenylidine glucoside) in the treatment of urinary bladder tumors. Neoplasma 24:411–414, 1977.
66. Schulman CC, Rozencweig M, Staquet M, et al. EORTC randomized trial for the adjuvant therapy of T1 bladder carcinoma. Eur Urol 2:271–273, 1976.
67. Reyes J, and Shimaoka K. Chemotherapy of thyroid carcinoma. Proc Am Assoc Cancer Res and ASCO 16:229, 1975.

COMMENTARY

Role of Routine Bone Scans in Operable Breast Cancer: An Opposing Viewpoint [1,2]

Janardan D. Khandekar [3,*]

Several studies have clearly demonstrated that radionuclide bone scans have increased sensitivity over radiographs in detecting skeletal metastases (1). Recently, Gerber et al (2) recommended that bone scans be performed routinely at the time of mastectomy, at 6-month intervals for 3 years, and then annually for 5 years to detect asymptomatic, radiographically occult skeletal metastases. These recommendations, if followed in the 90,000 breast cancer patients who are diagnosed annually, would cost between 150 and 200 million dollars a year. In these days of limited resources and spiraling medical costs, we should critically evaluate the usefulness of a given diagnostic procedure. In the present analysis, the Decision Matrix was used to analyze the usefulness of the bone scans in early breast cancer.

DATA BASE

The data on bone scans on which the Decision Matrix is based are derived from studies from this institution reported elsewhere (3) and from studies published in the literature (2–10) (*see* table 1). Only those studies with (*a*) a detailed description of methodology, (*b*) staging of patients by TNM classification (as adapted by the American Joint Committee for Cancer Staging and End-Results Reporting), (*c*) criteria of interpretation of scans, and (*d*) a full presentation of results were included in the analysis (2–10). A number of published reports were not included in our analysis because they lacked the above criteria. For example, Hoffman and Marty (11) reported results of bone scans in patients referred to them for "consideration for radiation." This obviously represents a biased series. There are at least four other published studies which report a high positive rate for scans (12–15). The following are the reasons for excluding these studies. Galasko (12) used ^{18}F as a scanning agent. Fluorine is known to have only 56% sensitivity as compared to technetium radiopharmaceuticals (16). This decreased sensitivity may have increased the specificity of the bone scans. Campbell et al (13) used the Manchester system for staging breast cancer. Roberts et al (14) correlated the results of their bone scans with the size of the primary tumor and did not give any consideration to the nodal status. Lentle and colleagues (15) did not analyze their results in relation to the stage of the breast cancer. However, it should be noted that these authors recommended scans only for T_3 and T_4 lesions.

The data were analyzed by the Decision Matrix technique. The principles of this type of analysis have been fully discussed by other investigators (17–19).

DEFINITIONS (*see also* Appendix I)

A true-positive (TP) ratio represents the proportion of positive tests in all patients who have the disease. It is expressed as:

$$\text{sensitivity} = \frac{\text{diseased subjects with positive test}}{\text{all diseased subjects tested}} \times 100.$$

The TP ratio therefore expresses the sensitivity of the test. The false-positive (FP) ratio is the proportion of positive tests in all patients who do not have the disease. The false-negative (FN) ratio is the proportion of negative tests in all patients with the disease. The true-negative (TN) ratio is the proportion of negative tests in all patients who do not have the disease and is expressed as:

$$\text{specificity} = \frac{\text{nondiseased subjects with negative test}}{\text{all nondiseased subjects tested}} \times 100.$$

This expresses the specificity of the examination.

The predictive value (PV) of a positive test is defined as the percentage of times a positive test will detect a diseased individual. This is expressed as:

$$\text{PV} = \frac{\text{number of diseased subjects (or proportion) with positive test}}{\text{total number (or proportion) of subjects with positive test}} \times 100$$

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DATA ANALYSIS

Ideally, a screening test should be very sensitive and specific, and yet inexpensive, simple, and unhazardous. Sensitivity is mainly determined by the FN rate, which is 0.16% for bone scans (1). A scan may be defined as FN when an abnormality is detected by roentgenograms, but not by scans. Bone scans are therefore very sensitive (99.84% sensitivity) in relation to the abnormalities detected by skeletal roentgenography. However, the sensitivity of bone scans is probably over-estimated, since their results have not been independently confirmed by other criteria, such as findings at autopsy. It is quite conceivable that if and when more sensitive methods for detecting skeletal metastases in vivo become available, the FN rate of bone scans will become much higher.

The major problem with the bone scan concerns its FP rate. A scan can be positive because of a number of benign osseous and soft tissue abnormalities (1). Various studies have reported an FP rate of between 3% and 64% (4,10). A recent report from the British Breast Group points to the unexplained variations in the bone scan results (20). In our studies, the FP rate was 15% (3).

Table 1 summarizes the results of bone scans from various studies. In stage I breast cancer, only 18 of 429 patients (4.2%) have positive bone scans. In stage II, 19 of 384 patients (4.95%) were positive for metastasis. If the results of stage I and II are added, and the 90 patients reported by Butzelaar et al (10) are included, 40 of 903 patients (4.43%) have positive bone scans. As defined earlier, the PV of a positive test is the percentage of times that a positive test will detect a diseased individual. Therefore, the PV of a positive test in stage I and II breast cancer patients is only 23.5% (*see* Appendix I). In other words, there is a possibility that three of four positive bone scans may be FP. In stage III breast cancer, 37 of 161 patients had positive bone scans. The PV of a positive scan in this group is therefore 66.5%.

TABLE 1.—Results of bone scans in breast cancer patients from various series*

Authors and ref No.	No. of patients with positive scans/No. of patients studied		
	Stage†		
	I	II	III
Baker et al, 4	1/27	0/36	10/31
Burkett et al, 3	1/80	2/59	2/23
Citrin et al, 5	6/49	5/26	0
Clark et al, 6	5/71	4/99	12/31
Davies et al, 7	2/98	1/56	6/38
Gerber et al, 2	2/73	0/37	5/12
Hammond et al, 8	0/1	3/29	3/13
O'Connell et al, 9	1/30	4/42	0/13
Total	18/429	19/384	37/161
% TP	4.2	4.95	22.98

*Combining stages I and II, 40 of 903 patients (4.43%) had positive bone scans (this includes series from Butzelaar et al [10], who combined stage I and II patients).

†Staging was adapted by the American Joint Committee for Cancer Staging and End-Results Reporting from the UICC-TNM classification.

DISCUSSION

The usefulness of a diagnostic test is directly proportionate to the prevalence of the target disease in the population. Factors influencing the FP rate of a test are usually constant and are unrelated to the disease itself. When the prevalence of a target disease is low, the PV of the test will be low, since the FP rate is constant (17). Since the yield of bone scans in stage I and II patients is low, and the FP rate is 15%, one may encounter a real clinical dilemma which may ensue regarding the management of patients whose positive scans are the only evidence of possible metastasis (9). The British Breast Group has also cautioned against using the scan results routinely in the management of early breast cancer (20). In these patients, a biopsy of the target bone will be required to confirm the diagnosis of bone metastasis.

On the other hand, in a group with a high prevalence of the disease and with an increased proportion of TP tests, the PV of a positive test will be high. Stage III breast cancer is a typical example of this Bayesian principle (21). Thirty-seven of 161 bone scans (22.9%) are positive in stage III disease. In this group, the PV of a positive test is 66.51%, and therefore bone scans are justified.

Recent studies have suggested that the routine use of multi-organ scans in the initial staging of potentially operable lung carcinoma and melanoma is not justified (22,23). Our own experience and that of others demonstrate that palpation is equivalent to or better than thyroid scans in selecting patients for surgery for thyroid cancer (24,25). All of these results indicate that scans and other expensive diagnostic procedures with a low yield and a significant rate of error in interpretation should be performed on a more selective basis in order to contain national health costs and, more importantly, to protect cancer patients from added economic burdens.

A recent report suggests that preoperative bone scans performed with ^{18}F accurately detected latent metastases years before they became clinically manifested (26). However, as pointed out earlier, ^{18}F bone scans have only 56% sensitivity compared to ^{99m}Tc, and this probably increased the specificity of ^{18}F scans.

A positive bone scan in a patient who otherwise has a localized disease signals a metastatic process therefore warranting systemic therapy. The major modalities for the treatment of metastatic breast cancer are chemoimmunotherapy and/or ablative and additive hormonal therapy. The acute side effects of chemotherapy, such as gastrointestinal toxicity, alopecia, and myelosuppres-

sion, are well-known. Chemotherapy is also known to be associated with a number of delayed side effects, including leukemia (27–29). Ablative endocrine therapy, such as adrenalectomy or hypophysectomy, is not without its morbidity. Although hormones such as tamoxifen do not produce severe toxicity, their long-term effects are unknown at present (30).

One can accept the high cost of treatment if early therapy is known to significantly alter the natural history of the underlying disease. Although it is well-known that patients with a good performance status, ie, those who are ambulatory, have a higher response rate when compared to those patients with a poor performance status, there is no evidence that early detection and therapy for metastatic breast cancer will alter the clinical course of the patient. It remains to be shown that breast cancer patients followed by aggressive, periodic testing survive significantly longer than patients followed conventionally by medical history, physical examination, and appropriate biochemical studies. It is interesting that in a recent review of 87 patients with recurrent breast cancer, only three had their disease detected with routine testing, ie, by chest roentgenogram or scan (31). In 79 such patients, the recurrence was heralded by the symptoms. Furthermore, in evaluating the impact of bone scans on patient survival, additional consideration will have to be given to some of the real and potential biases inherent in the evaluation of any screening test. These biases have been succinctly summarized by Bailar (32).

It is argued that a bone scan, performed at the time of mastectomy, will serve as baseline data, and therefore can be compared subsequently during the course of the patient's disease (2). It has been further suggested that serial bone scans may document occult progressive disease. Only two studies have looked at this scan-"conversion" (negative to positive) rate. Gerber et al (2) reported a 2-year, 21% scan-conversion rate for stage I breast cancer. However, the followup scans were performed in only 47 of the original patients. There was no discussion of whether these patients were studied at random or whether they had returned for evaluation of specific symptoms. McKillop et al (33) followed 75 patients with stage I and II breast cancer and observed a 20% scan-conversion rate, with a mean followup of 11 months. On the other hand, Robbins et al (34) reported that only 9.5% of patients with negative nodes developed bone metastases at 10 years. In stage I breast cancer, the 5- and 10-year recurrence rates are 21% and 24% respectively (35). Although it is conceivable that bone scans may detect skeletal metastases 2–3 years prior to its identification by roentgenograms, the high rate reported by Gerber et al (2) and McKillop et al (33) may not be representative of early breast cancer. Further studies are needed to determine the scan-conversion rate.

It can also be argued that patients receiving adjuvant chemotherapy should undergo scans every 6 months (27). This may be particularly indicated since a positive scan will dictate a change in chemotherapy, sparing the patients the toxic effects and cost of the ineffective regimen. Repeat bone scans will also expose patients to the hazards of radioactive substances. One bone scan with ^{99m}Tc gives approximately 0.1 rad of radiation to the bone marrow, and more to the bladder, stomach, and kidneys (36). Moreover, these patients are constantly exposed to a variety of other radiation, such as X-rays. Recent reports, although controversial, suggest that a single low exposure of 1.5 rems or long-term exposure to a very low level of radiation may be leukemogenic in certain populations (37–39). Patients with one malignancy are also susceptible to developing another tumor (40). Although it has not been shown that radiopharmaceuticals used for diagnosis are leukemogenic, it would be prudent to keep close surveillance on the patients who are given these agents.

In summary, the present analysis suggests that radionuclide bone scans are valuable in the following situations: (*a*) in symptomatic stage I and II breast cancer patients, (*b*) in stage III patients, (*c*) in patients receiving adjuvant chemoimmunotherapy, and (*d*) in patients in research protocols.

Appendix I.—Mathematic Expressions and Derivations Used in the Text

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity} = \frac{TN}{FP + TN} \times 100$$

where TP = true positivity, FN = false negativity, TN = true negativity, and FP = false positivity.

$$\text{Predictive value (PV)} = \frac{P \times Se}{(P \times Se) + (1-P)(1-SP)}$$

where P = disease prevalence, Se = sensitivity, and SP = specificity.

In stage I breast cancer, bone scans are positive in 4.2% of patients. Therefore,

$$PV = \frac{4.2 \times 99.84}{(4.2 \times 99.84) + (1-4.2)(1-85)} = 22.5\%.$$

In stage II breast cancer, bone scans are positive in 4.95% of patients. Therefore,

$$PV = \frac{4.95 \times 99.84}{(4.95 \times 99.84) + (1-4.95)(1-85)} = 25.74\%.$$

In stage III breast cancer, bone scans are positive in 22.98% of patients. Therefore,

$$PV = \frac{22.98 \times 99.84}{(22.98 \times 99.84) + (1-22.98)(1-85)} = 66.51\%.$$

REFERENCES

1. Lentle BC, Russell AD, Percy JS, et al. Bone scintiscanning updated. Ann Intern Med 84:297–303, 1976.
2. Gerber FH, Goodreau JJ, Kirchner PT, et al. Efficacy of preoperative and postoperative bone scanning in the management of breast carcinoma. N Engl J Med 293:300–303, 1977.
3. Burkett FE, Scanlon EF, Garces RM, et al. The value of bone scans in the management of breast cancer patients. Surg Gynecol Obstet, 1979. In press.
4. Baker FR, Holmes ER, Alderson PO, et al. An evaluation of bone scans as screening procedures for occult metastases in primary breast cancer. Ann Surg 186:363–368, 1977.
5. Citrin DL, Tormey DC, and Carbone PP. Implications of the ^{99m}Tc diphosphonate bone scan on treatment of primary breast cancer. Cancer Treat Rep 61:1249–1252, 1977.
6. Clark DG, Painter RW, and Sziklas JJ. Indications for bone scans in preoperative evaluation of breast cancer. Am J Surg 135:667–670, 1978.
7. Davies CJ, Griffiths PA, Preston BJ, et al. Staging breast cancer: role of bone scanning. Br Med J 2:603–605, 1977.
8. Hammond N, Jones SE, Salmon SE, et al. Predictive value of bone scans in an adjuvant breast cancer program. Cancer 41:138–142, 1978.
9. O'Connell MJ, Wahner HW, Ahmann DL, et al. Value of preoperative radionuclide bone scan in suspected primary breast carcinoma. Mayo Clin Proc 53:221–226, 1978.
10. Butzelaar RM, Van Dongen JA, Van Der Schoot JB, et al. Evaluation of routine preoperative bone scintigraphy in patients with breast cancer. Eur J Cancer 13:19–21, 1977.
11. Hoffman HC, and Marty R. Bone scanning: its value in the preoperative evaluation of patients with suspicious breast masses. Am J Surg 124:194–199, 1972.
12. Galasko CSB. The significance of occult skeletal metastases, detected by skeletal scintigraphy, in patients with otherwise apparently early mammary carcinoma. Br J Surg 62:694–696, 1975.
13. Campbell DJ, Banks AJ, and Oates GD. The value of preliminary bone scanning in staging and assessing the prognosis of breast cancer. Br J Surg 63:811–816, 1976.
14. Roberts JG, Gravelle IH, Baum M, et al. Evaluation of radiography and isotopic scintigraphy for detecting skeletal metastases in breast cancer. Lancet 1(7953):237–239, 1976.
15. Lentle BC, Burns PE, Dierich H, et al. Bone scintiscanning in the initial assessment of carcinoma of the breast. Surg Gynecol Obstet 141:43–47, 1975.
16. Barrett JJ, and Smith PHS. Bone imaging with ^{99m}Tc polyphosphate: a comparison with ^{18}F and skeletal radiography. Br J Radiol 47:387–392, 1974.
17. Vecchio TJ. Predictive value of a single diagnostic test in unselected populations. N Engl J Med 274:1171–1173, 1966.
18. McNeil BJ, Keeler E, and Adelstein SJ. Primer on certain elements of medical decision making. N Engl J Med 293:211–215, 1975.
19. Schwartz WB, Gorry GA, Kassirer JP, et al. Decision analysis and clinical judgment. Am J Med 55:459–472, 1973.
20. Preliminary statement by British Breast Group on bone scanning. Bone scanning in breast cancer. Br Med J 2:180–181, 1978.
21. Hall GH. The clinical application of Bayes' theorem. Lancet 2:555–557, 1967.
22. Ramsdell JW, Peters RM, Taylor AT, et al. Multi-organ scans for staging lung cancer. J Thorac Cardiovasc Surg 73:653–659, 1977.
23. Roth JA, Eilber R, Bennett LR, et al. Radionuclide photoscanning. Arch Surg 110:1211–1212, 1975.
24. Khandekar JD. A multidisciplinary approach to thyroid carcinoma developing in irradiated patients. Proc Am Assoc Cancer Res and ASCO 18:59, 1977.
25. Nelson RL, Wahner HW, and Gorman CA. Rectilinear thyroid scans as a predictor of malignancy. Ann Intern Med 88:41, 1978.
26. Sklaroff RB, and Sklaroff DM. Bone metastases from breast cancer at the time of radical mastectomy as detected by bone scan. Eight-year followup. Cancer 38:107–111, 1976.
27. Sieber SM, and Adamson RH. Toxicity of antineoplastic agents in man: chromosomal aberrations, antifertility effects, congenital malformations, and carcinogenic potential. Adv Cancer Res 22:57–144, 1975.
28. Khandekar JD, Kurtides ES, and Stalzer RC. Acute erythroleukemia complicating prolonged chemotherapy for ovarian carcinoma. Arch Intern Med 137:355–356, 1977.
29. Lerner HJ. Acute myelogenous leukemia in patients receiving chlorambucil as long-term adjuvant chemotherapy for stage II breast cancer. Cancer Treat Rep 62:1135–1138, 1978.
30. Legha SS, Davis HL, and Muggia FM. Hormonal therapy of breast cancer: new approaches and concepts. Ann Intern Med 88:69–77, 1978.
31. Winchester DP, Sener S, Khandekar JD, et al. Symptomatology as an indicator of recurrent or metastatic breast cancer. Cancer 43:956–961, 1979.
32. Bailar JC. Mammography: a contrary view. Ann Intern Med 84:77–83, 1976.
33. McKillop JH, Blumgurt LH, Wood CB, et al. The prognostic and therapeutic implications of the positive radionuclide bone scan in clinically early breast cancer. Br J Surg 65:649–652, 1978.
34. Robbins GF, Knapper WH, Barrie J, et al. Metastatic bone disease developing in patients with potentially curable breast cancer. Cancer 29:1702–1704, 1972.
35. Young RC, Lippman M, DeVita VT, et al. Perspectives in the treatment of breast cancer. Ann Intern Med 86:784–798, 1977.
36. Subramanian GI, and McAfee N. A new complex of ^{99m}Tc for skeletal imaging. Radiology 99:192–196, 1971.
37. Najarian T, and Colton T. Mortality from leukemia and cancer in shipyard nuclear workers. Lancet 1:1018, 1978.
38. Scanlon EF, Berk RS, and Khandekar JD. Post-irradiation malignancy: a symposium. *In* Current Problems in Cancer. Chicago, Ill, Year Book Medical Publishers, Inc, 1978, vol 3, No. 6.
39. Marx JL. Low-level radiation: just how bad is it? Science 204:160–164, 1979.
40. Moertel CG. Multiple primary malignant neoplasias. Cancer 40:1786–1792, 1977.

Phase I Evaluation of Tetrahydrouridine Combined With Cytosine Arabinoside [1,2,3]

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SUMMARY

In conventional clinical use, cytosine arabinoside (ara-C) is rapidly deaminated by pyrimidine nucleoside deaminase to the nontoxic compound uracil arabinoside. Tetrahydrouridine (THU) effectively inhibits this enzymatic degradation but is by itself nontoxic. This study demonstrates that concomitant administration of THU markedly increases the myelosuppressive potency of ara-C. When 25 or 50 mg/kg of THU iv and 0.1–0.2 mg/kg of ara-C iv are given daily × 5 days, they produce moderate-to-severe leukopenia and mild-to-moderate thrombocytopenia. A dose of 25 mg/kg of THU with 0.1 mg/kg of ara-C iv daily × 5 days appears appropriate for phase II studies; it produces myelosuppression equivalent to that produced by 3 mg/kg/day × 5 days of ara-C alone. No toxicity occurred with this combination that would not have been expected from ara-C given alone in an equitoxic dose. Although THU and ara-C produced a reduction in peripheral blood and bone marrow blast cells in eight of nine patients with acute leukemia, bone marrow remission did not occur in any of these heavily pretreated patients.

[Cancer Treat Rep 63:1245–1249, 1979]

Cytosine arabinoside (ara-C) is phosphorylated by deoxycytidine kinase to form arabinosylcytosine monophosphate; this undergoes sequential conversion to the diphosphate and triphosphate, the active metabolite of ara-C. It is also rapidly converted by pyrimidine nucleoside deaminase (PND) to uracil arabinoside, a biologically inert product (1). This rapid inactivation has led to the suggestion that the therapeutic efficacy of ara-C might be increased by blocking the degradative pathway. With the availability of tetrahydrouridine (THU) (fig 1), a potent inhibitor of PND, the experimental testing of this proposal has become feasible.

THU blocks deaminase activity in extracts of mouse kidney, rhesus monkey liver, and human liver (2). It also inhibits deamination of ara-C in intact mice, dogs, and rhesus monkeys (2–4). Philips and colleagues have demonstrated that THU potentiated the cytotoxic effects of ara-C upon bone marrow and gastrointestinal epithelium in dogs and monkeys, species which possess PND activity (unpublished observations). They failed to find comparable potentiation in the rat, a species which does not possess significant levels of PND.

Preclinical studies have also provided some evidence that the combination of THU and ara-C may produce antineoplastic effects beyond those expected with ara-C alone. Oral administration of THU increased the efficacy of oral ara-C against L1210 leukemia in mice, probably by inactivation of intestinal PND (5). Ip THU enhanced the therapeutic effect of ip ara-C in the ascitic, but not the solid, Sarcoma 180 tumor (6). Chou and colleagues (7) have demonstrated that THU increases the capacity of human myelocytic and monomyelocytic leukemic cells to synthesize arabinosylcytosine triphosphate in vitro.

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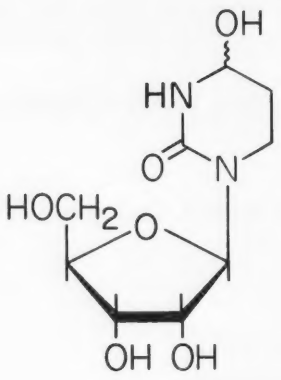

FIGURE 1.—Structural formula of THU.

This latter observation was one of the persuasive factors which led us to initiate a clinical trial. THU alone has been shown to be nontoxic when given in large iv doses (up to 1 g/kg) to dogs and monkeys (8).

The clinical pharmacologic studies of Kreis and colleagues (9) that accompanied this phase I trial demonstrated that when THU was administered at a dose of 10, 25, or 50 mg/kg 15 minutes before a dose of ara-C, the resultant blood levels of ara-C were as much as four times the comparable blood level when ara-C was given alone. These pharmacokinetic studies predicted that THU would potentiate the biologic activity of ara-C in man; the present report documents that the expected potentiation did occur.

PATIENTS AND METHODS

THU was supplied for this study by the National Cancer Institute (Bethesda, Md) as a bulk, hygroscopic powder and stored at –10°C. It was dissolved at a concentration of 50 mg/ml in 5% dextrose and water and sterilized by filtration. The drug was administered by iv injection 15 minutes before ara-C. Solutions not used immediately were stored at 4°C for up to 48 hours and then discarded. Ara-C (Cytosar) was obtained in 100-mg vials as a white powder from the Upjohn Company (Kalamazoo, Mich). Five milliliters of bacteriocidal water was used as a diluent to an initial concentration of 20 mg/ml. This was refrigerated for up to 2 days prior to use.

Patients selected for this study had biopsy-proven advanced, nonresectable neoplastic disease for which no effective conventional therapy was available. All patients had a life expectancy of ≥ 1 month and a Karnofsky performance status of ≥ 30. All patients with solid tumors had a wbc count of ≥ 4000/mm^3 and a platelet count of ≥ 200,000/mm^3. Informed consent for the drug trial was obtained from all participants.

A total of 49 adult patients were enrolled in this study. The median age was 50 years. Twenty-eight patients had solid tumors and 21 had lymphoma or leukemia. Nearly all patients had had prior chemotherapy and/or radiotherapy. The 12 patients with leukemia and one of the nine patients with lymphoma had received prior ara-C.

The study was performed in two stages; the first stage consisted of THU given alone to evaluate its toxicity. The initial dose was 10 mg/kg/day iv with subsequent doses increased stepwise (10, 20, 40, and 50 mg/kg/day) up to 50 mg/kg/day for up to 20 days. In the second stage, ara-C was given with THU. THU was given iv 15 minutes prior to the iv dose of ara-C because metabolic studies by Kreis et al in man (9) and El Dareer and colleagues in animals (10) had demonstrated that THU was rapidly excreted. For maximal effect therefore, ara-C needed to be administered before a significant drop in the plasma-THU level had occurred. The initial dose was 10 mg/kg for THU and 0.0025 mg/kg for ara-C. Incremental escalation of the ara-C dose was performed by a modified Fibonacci progression until some biologic effect was seen. Each dose level of ara-C was given for 5–7 days; the courses were cumulative. With the demonstration of a biologic effect of ara-C when given with 10 mg/kg of THU, the dose-finding procedure was repeated at 25 and 50 mg/kg using 5-day courses at 28-day intervals.

All patients were hospitalized and monitored for evidence of hematopoietic, gastrointestinal, hepatic, renal, and central nervous system toxicity. Daily cbcs were performed and twice weekly 12-channel screening profiles were obtained.

RESULTS

Tetrahydrouridine Alone

Four patients received THU alone; the first two patients began treatment at 10 mg/kg/day. When toxicity did not appear after 3–4 days, the dose of THU was increased stepwise as previously described. Subsequent patients began treatment at 20 mg/kg/day; one patient received 50 mg/kg/day for 23 days. The average total dose was 750 mg/kg; neither toxicity nor therapeutic response was observed.

Tetrahydrouridine and Cytosine Arabinoside

Myelosuppression

In preliminary studies THU was given at a dose of 10 mg/kg/day with escalating and cumulative doses of ara-C for 5–7 days from 0.0025 to 0.4 mg/kg. Myelosuppression did not occur until the daily dose of ara-C reached 0.1–0.4 mg/kg (data not shown). Detailed myelotoxicity stud-

TABLE 1.—Hematopoietic toxicity of daily THU (25 and 50 mg/kg) and ara-C therapy: dose-effect relationships

Ara-C dose (mg/kg)	No. of patients at risk	No. of evaluable courses	No. of courses with wbc count nadir (cells × 10^3/mm^3) of—			No. of patients at risk	No. of evaluable courses	No. of courses with platelet count nadir (cells × 10^3/mm^3) of—		
			>4.0	2.0–3.9	<2.0			>150	80–150	<80
25-mg/kg THU dose										
0.02	2	2	2	0	0	2	2	2	0	0
0.04	3	3	2	0	1	3	3	2	0	1
0.08	3	4	0	4	0	3	4	0	2	2
0.2	5	6	0	4	2	5	6	0	5	1
0.3	5	6	1	0	5	5	4	0	0	4
0.4	2	2	0	1	1	2	2	0	0	2
0.6	2	2	0	0	2	2	2	0	0	2
50-mg/kg THU dose										
0.02	4	4	4	0	0	4	4	4	0	0
0.05	3	3	2	1	0	3	3	2	0	1
0.08	6	7	2	3	2	6	7	3	1	3
0.1	2	2	0	0	2	2	2	1	0	1
0.2	6	6	2	2	2	6	6	0	2	4
1.0	3	4	0	2	2	3	3	0	0	3

ies were carried out in patients with normal marrow reserves using 5-day courses of 25 or 50 mg/kg of THU and a daily × 5 dose of ara-C. The results of these studies are summarized in table 1. Table 2 illustrates the time of nadir and recovery from these toxic effects. Thrombocytopenia occurred first, reaching a nadir 13 days following initiation of therapy. Recovery required a median of 15 and 18 days for mild and severe thrombocytopenia, respectively. While the platelet count was recovering, the leukocyte count fell, reaching a median nadir at 19 and 16 days for mild and severe leukopenia, respectively. The median time to leukocyte recovery from the nadir was 3 and 5 days, respectively.

Eleven continuous infusion trials of THU and ara-C were performed in solid tumor patients. THU at a dose of 50 mg/kg and ara-C at a dose of 0.05 mg/kg daily × 5 days produced leukopenia (≤ 3000 cells/mm^3) and thrombocytopenia (≤ 20,000 cells/mm^3) in only one of six trials. Infusion of 50 mg/kg of THU and 0.1 mg/kg of ara-C daily × 5 days produced myelosuppression in each of five patients; the median nadirs were 1200 leukocytes/mm^3 and 75,000 platelets/mm^3.

Other Toxic Effects

Aside from myelosuppression, drug toxicity was relatively minor. A few patients (5%) experienced mild nausea immediately after both drugs were given. Two patients developed drug-related conjunctivitis which resolved spontaneously. Two patients manifested a self-limited erythematous, nonpruritic skin eruption. Symptomatic hypotension occurred in one patient at a THU dose of 50 mg/kg (with and without subsequent ara-C) but was not observed at a dose of 25 mg/kg. At higher doses of ara-C used in leukemic patients, one patient developed diffuse gastritis with bleeding. Drug-induced hepatic, renal, or central nervous system dysfunction was not observed in this series.

Therapeutic Activity

In this preliminary study, only slight therapeutic activity was noted. A patient with metastatic jejunal carcinoma had a minor response, as did two of five evaluable patients with diffuse histiocytic lymphoma. A marked reduction in the numbers of circulating blast cells and in the degree of bone marrow infiltration occurred in eight of nine adequately treated leukemic patients treated with THU and ara-C; these included the two patients with acute myelomonocytic leukemia, the three with acute lymphocytic leukemia, and the three with chronic myelogenous leukemia in blast cell crisis (the patient without this therapeutic effect had erythroleukemia). Bone marrow remission was not obtained in any of these patients; all had received ara-C as part of their prior therapy. Two patients developed clearcut resistance to further therapy with THU and ara-C, with increasing numbers of bone marrow and circulating blast cells, in spite of continuing drug administration.

TABLE 2.—Myelosuppression produced by the combined use of THU and ara-C

	No. of courses	Median treatment Day for—	
		Nadir	Recovery
Leukopenia			
Mild*	18	19	22
Severe†	22	16	21
Thrombocytopenia			
Mild*	10	13	15
Severe†	29	13	18

*Wbc count nadir of ≥ 2000/mm^3; platelet count nadir of ≥ 80,000/mm^3.

†Wbc count nadir of < 2000/mm^3; platelet count nadir of < 80,000/mm^3.

DISCUSSION

Ara-C is an important agent in the therapy for myelomonocytic leukemia in man. One approach toward improving the therapeutic index of this drug has centered upon blocking its in vivo deamination by the use of THU. The clinical pharmacologic studies of Kreis and colleagues (9) have demonstrated the ability of THU to prolong and elevate plasma levels of ara-C beyond those controls receiving ara-C alone.

The present study establishes the potentiation in man of the biologic activity of ara-C by THU. With 5-day courses, 25 or 50 mg/kg of THU and 0.1–0.2 mg/kg of ara-C produced appreciable but tolerable myelosuppression. The 25-mg/kg THU dose and the 0.1-mg/kg ara-C dose are equivalent to a 5-day course of 3 mg/kg of ara-C alone (11). It would seem an appropriate choice for phase II studies. Not surprisingly, no toxicity developed other than that which has been previously seen with ara-C alone.

No difference was noted between administering daily injections and continuous infusions. This indicates that the effect of THU in providing prolonged effective levels of ara-C is maximal and cannot be augmented by further attempts to provide effective levels of continuous infusion of both agents. This is in marked contrast to the situation which prevails when ara-C is given alone; in that circumstance, continuous infusions of ara-C result in much more biologic activity than when equivalent doses are given in single daily iv injections (12,13).

No useful therapeutic effects were noted in this toxicologic study. Future investigations with patients who have been less heavily pretreated are needed to rigorously examine the therapeutic potential of this combination. In addition to patients with leukemia, disease-

oriented trials might also properly focus on gastrointestinal adenocarcinomas, since other studies have indicated that ara-C alone (13), or a related compound, 2,2′-anhydro-1-β-D-arabinofuranosyl-5-fluorocytosine (14), may be of some value in these malignancies.

REFERENCES

1. CREASEY WA, PAPAC RJ, MARKIW ME, ET AL. Biochemical and pharmacological studies with 1-β-D-arabinofuranosylcytosine in man. Biochem Pharmacol 15:1417–1428, 1966.
2. CAMIENER GW. Studies of the enzymatic deamination of aracytidine. V. Inhibition in vitro and in vivo by tetrahydrouridine and other reduced pyrimidine nucleosides. Biochem Pharmacol 17:1981–1991, 1968.
3. WHITE VM, MELLETT LB, and EL DAREER SM. The effect of tetrahydrouridine (H_4U) on the metabolism of arabinosylcytosine (ara-C) in tumor-bearing mice. Pharmacologist 17:202, 1975.
4. EL DAREER SM, MULLIGAN LT, JR, WHITE V, ET AL. Distribution of [^{3}H]cytosine arabinoside and its products in mice, dogs, and monkeys and effect of tetrahydrouridine. Cancer Treat Rep 61:395–407, 1977.
5. NEIL GL, MOXLEY TE, and MANAK RC. Enhancement by tetrahydrouridine of 1-β-D-arabinofuranosylcytosine (cytarabine) oral activity in L1210 leukemic mice. Cancer Res 30:2166–2172, 1970.
6. KREIS W, HESSION C, SORICELLI A, ET AL. Combinations of tetrahydrouridine and cytosine arabinoside in mouse tumors. Cancer Treat Rep 61:1355–1364, 1977.
7. CHOU T-C, ARLIN Z, CLARKSON BD, ET AL. Metabolism of 1-β-D-arabinofuranosylcytosine in human leukemic cells. Cancer Res 37:3561–3570, 1977.
8. GOLDENTHAL EI, COOKSON KM, GEIL RG, ET AL. Preclinical toxicologic evaluation of tetrahydrouridine (NSC-112907) in beagle dogs and rhesus monkeys. Cancer Chemother Rep Part 3, vol 5(1):15–16, 1974.
9. KREIS W, WOODCOCK TM, GORDON CS, ET AL. Tetrahydrouridine: physiologic disposition and effect upon deamination of cytosine arabinoside in man. Cancer Treat Rep 61:1347–1353, 1977.
10. EL DAREER SM, WHITE V, CHEN FP, ET AL. Distribution of tetrahydrouridine in experimental animals. Cancer Treat Rep 60:1627–1631, 1976.
11. TALLEY RW, and VAITKEVICIUS VK. Megaloblastosis produced by a cytosine antagonist 1-β-D-arabinofuranosylcytosine. Blood 21:352–362, 1963.
12. BURKE PJ, SERPICK AA, CARBONE PP, ET AL. A clinical evaluation of dose and schedule of administration of cytosine arabinoside (NSC 63878). Cancer Res 28:274–279, 1968.
13. FREI E III, BICKERS JN, HEWLETT JS, ET AL. Dose schedule and antitumor studies of arabinosylcytosine (NSC 63878). Cancer Res 29:1325–1332, 1969.
14. ALBERTO P, GANGJI D, KENIS Y, ET AL. Phase II study of anhydro-ara-5-fluorocytidine (AAFC). Proc Am Assoc Cancer Res and ASCO 18:231, 1977.

Interaction of Thoracic Irradiation and Chemotherapy on Local Control and Survival in Small Cell Carcinoma of the Lung[1]

James D. Cox,* Roger Byhardt, Ritsuko Komaki, J. Frank Wilson, Joseph A. Libnoch, and Richard Hansen[2]

SUMMARY

The records of all patients with small cell carcinoma of the lung seen in the Division of Therapeutic Radiology at The Medical College of Wisconsin from 1971 through 1977 were reviewed. Of 69 patients (34 with limited disease and 35 with extensive disease), 35 received irradiation only, nine received local plus total-body irradiation, and 25 received irradiation plus chemotherapy. In spite of the prompt response of the tumor, 16 of 35 patients who were only irradiated failed locally. Two patients who received local plus total-body irradiation failed in the chest. Only four of 25 irradiation plus chemotherapy patients failed locally in spite of much lower doses of thoracic irradiation. Limited-disease patients who had local control by irradiation had a slight increase (29 vs 22 weeks) in median survival. Patients receiving irradiation plus chemotherapy (three with limited and 22 with extensive disease) survived for a median of 56 weeks if the local tumor was controlled and for a median of 28 weeks if there was local failure. Regardless of therapy, survival beyond the median is greatly influenced by local tumor control.

[Cancer Treat Rep 63:1251–1255, 1979]

Small cell carcinoma of the lung is a leading cause of death from cancer in the US. Since it accounts for one fifth of all broncho-pulmonary malignant tumors and is rarely cured, it is responsible for nearly 20,000 deaths annually. Thus, it causes more deaths from malignant disease than all but squamous cell carcinoma and adenocarcinoma of the lung and adenocarcinoma of the large bowel and breast (1).

In the past 5 years, a great surge of clinical research has been directed toward small cell carcinoma. The striking responsiveness of this disease, both to irradiation and to several cytotoxic drugs, has led to the emergence of many regimens combining thoracic and cranial irradiation with combination chemotherapy. Such combinations can be accompanied by significant morbidity and mortality (2). Since it is now widely accepted that chemotherapy is always indicated in this disease, it is essential to determine the dose of irradiation necessary to control the tumor with minimal morbidity and, indeed, whether irradiation is mandatory when chemotherapeutic combinations are so effective.

Previous studies from The Medical College of Wisconsin (3) suggested that the dose of irradiation could be reduced greatly without a sacrifice in local tumor control, especially when combined with cyclophosphamide, Adriamycin, and vincristine (CAV). However, it was recognized that recurrence might only be delayed since the observation period of many patients treated with irradiation and CAV was relatively short. Furthermore, since there was no apparent improvement in median survival when the thoracic tumor was controlled, the importance of *any* thoracic irradiation could be questioned.

The following report is an update of The Medical Col-

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lege of Wisconsin experience. It addresses the questions of long-term control with irradiation plus chemotherapy, and the importance of local, ie, intrathoracic, tumor control to survival.

MATERIALS AND METHODS

From 1971 through 1977, 69 patients with small cell carcinoma of the lung were treated in the Division of Therapeutic Radiology of The Medical College of Wisconsin-affiliated hospitals. From 1971 through 1974, patients with limited disease (ie, involvement limited to the primary tumor, ipsilateral hilar or supraclavicular nodes, and ipsilateral or bilateral mediastinal nodes) received irradiation to the primary and nodal disease. From 1975 through 1977, all patients, except three who refused, received thoracic irradiation plus chemotherapy.

In 1976–1977, patients with limited disease were randomized to local plus total-body irradiation (TBI) versus local irradiation plus CAV. (Patients with positive bone marrow biopsies, but no other evidence of metastasis, were included in this trial.) Patients with extensive disease were treated according to a phase II protocol of local irradiation and CAV. In all patients, local irradiation was started immediately, while the initial evaluation was completed, and TBI or chemotherapy was started during or immediately following irradiation. Local irradiation consisted of a minimum tumor dose of 3000 rads in ten fractions in 2 weeks. TBI consisted of a midsagittal plane dose of 100 rads in ten fractions in 2 weeks. Both local irradiation and TBI were administered five times per week.

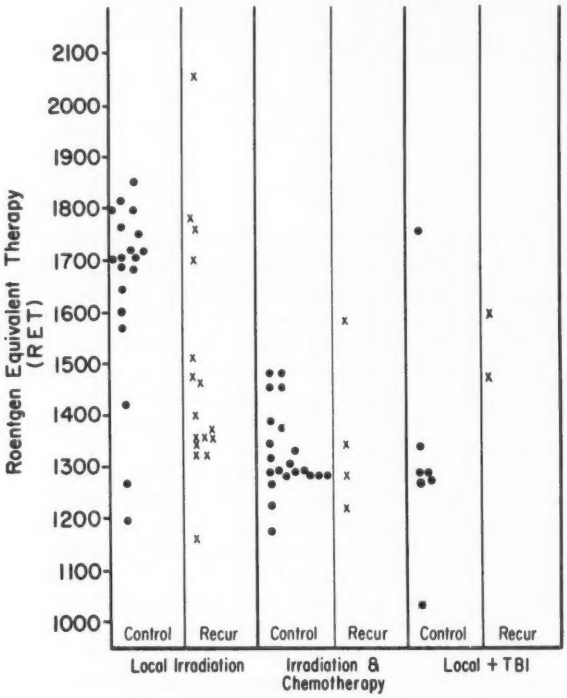

FIGURE 1.—Control of thoracic tumor by treatment group and biologic dose.

Patients were followed jointly by medical and radiation oncologists. Local failure was assessed by serial chest roentgenograms and tomograms, or computerized tomography, when indicated. If there was persistent evidence of tumor within the volume encompassed by the local irradiation, whether in the lung, mediastinum, or supraclavicular areas, it was considered local failure; similarly, any regrowth of tumor within these areas, at any time, was also considered failure. If autopsies were performed, histologic evidence of residual disease within the field of thoracic irradiation was considered to be local persistence or recurrence. No patient was lost to followup.

Variations of dose, fractionation, and time have been reduced to the Nominal Standard Dose (NSD) by the formula $NSD = \frac{D}{N^{0.24} T^{0.11}}$ where D = total dose (rads), N = number of fractions, and T = number of days for the course of treatment (4). The modification related to partial tolerance was not used. The NSD and all formulae which reduce a course of irradiation to a single number are considered to be approximations of *biologic doses* which may suggest trends, but the precise numerical values are of little importance.

RESULTS

A more detailed description and the results of the prospective trials have been presented elsewhere.[3] In brief, all patients who received local irradiation plus TBI developed progressive disease but were immediately given CAV. Their bone marrow tolerance and subsequent response to chemotherapy were not altered by the TBI.

Characteristics of the three treatment groups are shown in table 1. The groups are comparable except for the high proportion of patients with limited disease who received irradiation only and the high proportion with extensive disease who received irradiation plus chemotherapy. Four patients who received local irradiation plus TBI had positive bone marrow biopsies as the only evidence of extensive disease.

Results of the thoracic irradiation are shown in figure 1 and table 2. Local control improved to a striking degree with the use of chemotherapy, in spite of much lower

[3]Byhardt RW, Stein RS, Cox JD, et al. Total body irradiation as a systemic adjuvant for small cell carcinoma of the lung. Presented at the American Society of Therapeutic Radiologists, Los Angeles, Calif, Nov 1978. In press in *Int J Radiat Oncol Biol Phys.*

TABLE 1.—Patient characteristics by treatment group of small cell carcinoma of the lung at The Medical College of Wisconsin: 1971–1977

Characteristic	Treatment group		
	Local irradiation	Local irradiation + TBI	Local irradiation + chemotherapy
No. of patients	35	9	25
Males/females	28/7	7/2	17/8
Age in yrs			
Range	47–81	46–70	42–76
Median	60	60	58
No. of patients with limited disease/No. with extensive disease	26/9	5/4	3/22

biologic doses of irradiation. Fourteen of 16 (88%) disease recurrences after local irradiation were detected within 24 weeks. With TBI plus chemotherapy, three recurrences were detected within 24 weeks but the other recurrences were found at 35, 37, and 40 weeks. This suggests a delay in the appearance of recurrences, but there are too few local failures to draw firm conclusions. Since the median survival of the TBI plus chemotherapy patients is > 40 weeks, it is unlikely that more local recurrences will be seen.

The influence of local control of the thoracic tumor on survival is analyzed in table 3. The median survival more than doubled with the addition of chemotherapy. The

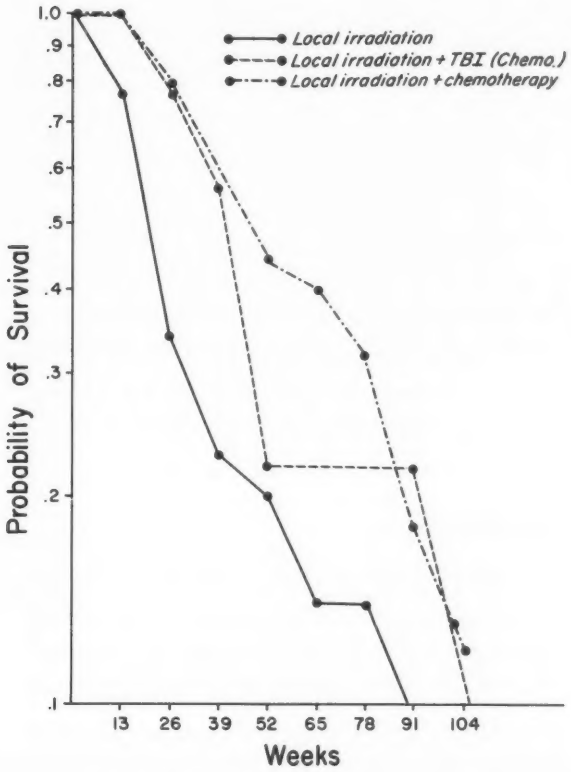

FIGURE 2.—Survival by treatment group.

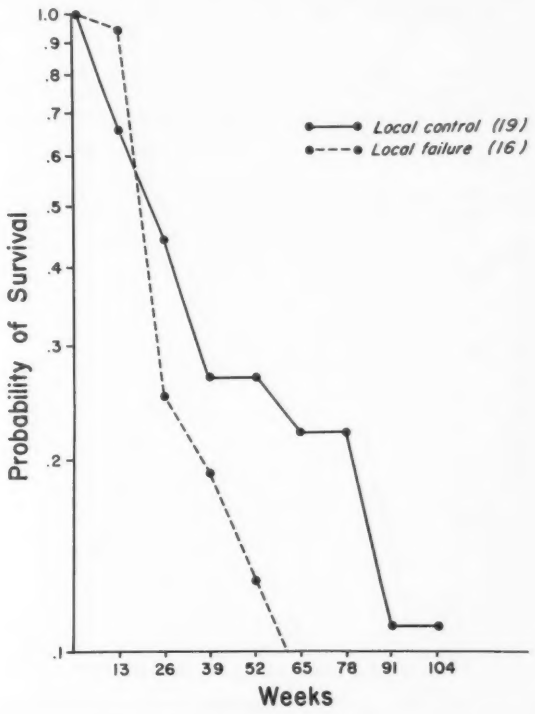

FIGURE 3.—Effect of local control on survival after irradiation.

TABLE 2.—Local tumor control by treatment group of small cell carcinoma of the lung at The Medical College of Wisconsin: 1971–1977

	Treatment group		
	Local irradiation	Local irradiation + TBI	Local irradiation + chemotherapy
Biologic dose in rads (NSD)*			
Range	1165–2055	1029–1753	1172–1577
Median	1647	1281	1290
No. of patients with—			
Local tumor control (limited disease/extensive disease)	19 (13/6)	7 (4/3)	21 (3/18)
Local failure (limited disease/ extensive disease)	16 (13/3)	2 (1/1)	4 (0/4)

**See* Materials and Methods section for explanation of NSD.

improvement in survival is even more pronounced if only extensive-disease patients are considered: 12 weeks for irradiation alone versus 43 weeks for local irradiation and chemotherapy. There is little difference in median survival with or without local control in limited-disease patients treated with irradiation alone (median survival independent of local control is 24 weeks). If all irradiation plus chemotherapy patients are considered together (three with limited and 22 with extensive disease), the median survival is 56 weeks with control of the thoracic tumor and 28 weeks with local failure. Thus, there is improvement in median survival with local tumor control independent of the increased survival with chemotherapy.

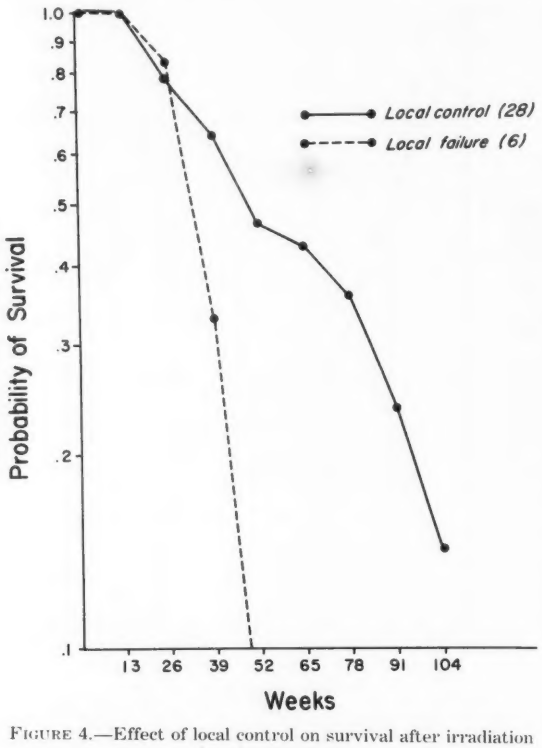

FIGURE 4.—Effect of local control on survival after irradiation plus chemotherapy.

However, the survival curves more convincingly demonstrate the importance of local tumor control. Figure 2 shows the survival for the three treatment groups. As noted previously, patients who received TBI actually constitute a delayed-chemotherapy group. Figures 3 and 4 show the influence of local tumor control on survival beyond the median with irradiation alone and with irradiation plus chemotherapy (including the TBI plus chemotherapy patients).

An analysis on January 1, 1979, showed that four patients are alive. A 69-year-old man with limited disease received only thoracic irradiation and has no evidence of disease at 69 months. A 42-year-old woman with disseminated disease who received irradiation plus CAV has no evidence of disease 25 months after the first day of irradiation; chemotherapy was discontinued after the 12th month. Two men, 49 and 59 years old, are alive with evidence of disease at 18 and 20 months.

DISCUSSION

A review of phase III trials in small cell carcinoma (3) revealed a wide range of doses of thoracic and cranial irradiation. The data presented above indicate that very modest doses, when combined with effective chemotherapy, suffice to control even bulky disease. These data are limited to a single continuous course of irradiation plus concomitant and subsequent chemotherapy and may not apply to "split-course" irradiation. At the present time, patients with small cell carcinoma treated at The Medical College of Wisconsin receive 3750 rads in 15 fractions in

TABLE 3.—Local tumor control vs median survival (wks) of small cell carcinoma of the lung at The Medical College of Wisconsin: 1971–1977

Local tumor control in patients with—	Median survival in wks of group treated with—		
	Local irradiation	Local irradiation + TBI	Local irradiation + chemotherapy
Limited + extensive disease	22 (35)*	43 (9)	48 (25)
Limited disease			
Primary control	29 (13)	29 (4)	66 (3)
Primary failure	22 (13)	43 (1)	— (0)
Extensive disease	12 (9)	43 (4)	43 (22)

*Nos. in parentheses = No. of patients.

3 weeks to areas of known disease and 2500 rads in ten fractions in 2 weeks to areas irradiated prophylactically. All patients also receive combination chemotherapy.

Analysis of the effect of local, ie, thoracic, tumor control reveals little influence on *median* survival when irradiation alone is used. This is consistent with the studies of failure pattern and cause of death by cell type (5) which showed that local failure was a much less important problem with small cell carcinoma than with squamous cell carcinoma. Most patients with small cell carcinoma die with widespread dissemination. There is no question that chemotherapy should be started as soon as the initial evaluation is complete.

However, control of the thoracic tumor clearly affects *survival beyond the median* and is a sine qua non for cure of these patients. The unanswered question at present is whether chemotherapy alone is sufficient to control even bulky disease in the chest. Studies are underway at our institution to answer that question.

REFERENCES

1. Cancer Facts and Figures, 1977. New York, NY, American Cancer Society, 1978, p 10.
2. BRERETON HD, KENT HC, and JOHNSON RE. Chemotherapy and radiation therapy for small cell carcinoma of the lung: a remedy for past therapeutic failure. *In* Lung Cancer: Progress in Therapeutic Research (Muggia F, and Rozencweig M, eds). New York, NY, Raven Press, 1979, pp 575–586.
3. COX JD, BYHARDT RW, WILSON JF, ET AL. Dose-time relationships and the local control of small cell carcinoma of the lung. Radiology 128:205–207, 1978.
4. ELLIS F. The relationship of biological effect to dose-time-fractionation factors in radiotherapy. *In* Current Topics in Radiation Research (Ebert M, and Howard A, eds). Amsterdam, The Netherlands, North Holland Publishing Co, 1968, pp 357–397.
5. COX JD, YESNER R, and PETROVICH Z. Influence of cell type on failure pattern after irradiation for locally advanced carcinoma of the lung. Cancer. In press.

High-Dose BCNU Therapy With Autologous Bone Marrow Infusion: Preliminary Observations [1,2]

Gary Spitzer,* Karel A. Dicke, Dharmvir S. Verma, Axel Zander, and Kenneth B. McCredie [3,4]

SUMMARY

Nine patients with solid malignancies and extensive prior treatment received high-dose BCNU therapy (600–750 mg/m^2) with autologous bone marrow support; following this treatment hematopoietic recovery was studied. The only significant nonhematopoietic toxicity was a probable case of BCNU-induced pulmonary toxicity in a patient who had received massive amounts of prior chemotherapy and chest irradiation. The marrow aspirations prior to cryopreservations had revealed a hypoplastic marrow in four of nine patients. Despite using marrow exposed to prior chemotherapy, neutropenia beyond Day 40 after BCNU therapy was not observed in any patient. One patient did not develop neutropenia of $< 1.5 \times 10^9$ cells/liter and five patients did not develop neutropenia of $< 0.5 \times 10^9$ cells/liter. A partial response was observed in one patient and less than partial responses were observed in two other patients. Autologous bone marrow infusion may modify the neutropenia of high-dose BCNU therapy.

[Cancer Treat Rep 63:1257–1264, 1979]

Hematopoietic stem cells in the bone marrow of several species can be stored at low temperatures in liquid nitrogen using dimethyl sulfoxide (DMSO), glycerol, or other agents as cryoprotective agents. Marrow thus preserved can rescue mice from the lethal hematopoietic toxicity of an LD100 dose of irradiation (1,2). Autologous bone marrow transplantation (ABMT) can also protect against lethal irradiation in both dogs and primates (3–6). There have been several studies in preclinical models on the ability of frozen bone marrow to protect against chemotherapy toxicity. Stored isogeneic marrow infusions have been reported not to protect against the lethal effects of mechlorethamine but to be lifesaving in dogs after thioTEPA at doses of 5–6 mg/kg or high-dose cyclophosphamide (7–9).

Most studies in man using high-dose chemotherapy followed by ABMT have been ambiguous. Because of the relatively low drug doses used, the small numbers of cells usually infused, and the variable freezing technologies, it has been difficult to demonstrate clearly that frozen or nonfrozen autologous bone marrow can modify the hematopoietic toxicity of mustard hydrochloride, monomustine dihydrochloride, and high-dose irradiation to areas of tumor involvement (10–13). More recently, Appelbaum et al (14) and Gorin et al (15) described rapid hematopoietic recovery using ABMT after high-dose combination chemotherapy. Our studies in acute leukemia have proven the ability of frozen autologous bone marrow to reconstitute the hematopoietic system after lethal doses of total-body irradiation and high-dose chemotherapy (16–19).

With this recent proven ability of marrow to rescue from lethal doses of marrow-suppressive agents, we initiated studies to determine the feasibility of using high-dose BCNU with ABMT in a group of nine patients whose marrow was collected after failure to respond to prior extensive chemotherapy. This paper describes our initial experience with alteration of BCNU-induced hematopoietic toxicity by ABMT in patients with solid malig-

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[4]We greatly appreciate Lee Merriweather for her efforts in typing this manuscript.

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nancies who had failed to respond to extensive prior chemotherapy.

MATERIAL AND METHODS

Informed Consent

This protocol was approved by the Institutional Human Surveillance Committee and all patients signed an informed consent that they were aware of all possible complications of aspiration and storage of marrow and administration of chemotherapy.

Definition of Response

Partial response was defined as a decrease of $\geq 50\%$ in the product of the largest perpendicular diameters of all measurable disease for at least 4 weeks. A less than partial response was defined as a decrease of $< 50\%$ but $> 25\%$ in all measurable disease or either greater than partial or complete disappearance of some measurable disease without response elsewhere, eg, subcutaneous disease response without a simultaneous partial response within the lung. Stable disease refers to no evidence of disease progression or a small tumor reduction for at least 4 weeks.

Drug Doses

BCNU was given by slow iv infusion over 30–60 minutes in 250 ml of dextrose and water. Each patient received a total dose of 600–750 mg/m^2 over a 7-day period. Patients 1 and 2 received 300 mg/m^2 on the first and seventh days respectively. Because of the absence of severe nonhematopoietic side effects, patients 3–7 received 300 mg/m^2 of BCNU and patients 8 and 9 received 375 mg/m^2 of BCNU on 2 consecutive days (Days 1 and 2). Patient 4 also received hydroxyurea at a dose of 1 g/m^2 for 7 consecutive days starting on Day 1. At least 5 days after the last BCNU dose, bone marrow was thawed and infused.

Multiple Aspirations, Storage, and Marrow Transfusion

An approximate volume of 1500 ml was aspirated from both posterior iliac spines and crests and collected into glass bottles containing 50 ml of Hanks' balanced salt solution (HBSS) buffered with 4-(2-hydroxyethyl)-1-piperazinethane sulfonic acid (HEPES), 0.5 ml of deoxyribonuclease (3.33 mg/ml), and 2 ml of heparin (5000 units/ml) per 1000 ml of bone marrow collection. Cells were transferred to large glass centrifuge tubes (80 ml/tube) and centrifuged in a refrigerated IEC centrifuge (2000 rpm for 10 minutes); the buffy coat was obtained and filtered through a 40μ G-2 glass filter. The cells were then further centrifuged, the supernatant was removed, and the cells were kept at 4°C until resuspension in freezing media.

Equal volumes of cells in HBSS and HEPES were mixed with equal volumes of 20% DMSO and 40% fetal calf serum to give a final concentration of 10% DMSO and 20% serum. Cells were stored in 5-ml polypropylene plastic ampules at a concentration of 200×10^6/ml and a total of 800×10^6/ampule. Cells were frozen at 1°C/minute from 4°C to –40°C and then rapidly frozen until approximately –80°C in a Cryoson programmed freezer (Cryoson Midden, Beemster, Holland). The ampules were then stored at –192°C in the liquid phase of a liquid nitrogen freezer. At the time of infusion, the ampules were removed from the storage tank, rapidly thawed in a 50°C water bath, and diluted stepwise to 10 times their original volume with HBSS. Cells were then further washed with this solution to remove DMSO and serum, filtered through a G-2 glass filter, and infused iv in an approximate volume of 250 ml over a period of 30–60 minutes. All patients were premedicated iv with 50 mg of diphenhydramine hydrochloride and 250 mg of methylprednisolone. Further details on these procedures can be found in other publications (16–20).

RESULTS

Nine patients, six males and three females, were treated with high-dose BCNU and ABMT. The diagnoses, prior therapy, extent of disease, drug doses, and responses are given in table 1. There were six cases of disseminated malignant melanoma and three cases of oat cell carcinoma of the lung. All patients had failed to respond to prior chemotherapy and most had received chemotherapy for extended periods of time (median, 10 months; range, 4–23 months) before multiple marrow aspiration and storage. Because of the extensive prior therapy, four patients (Nos. 1, 3, 5, and 7) had hypoplastic bone marrows at the time of marrow collection and storage. Their clot section cellularity was only 15% compared to $\geq 30\%$ in normal individuals (21). The first two patients entered in this program received 300 mg/m^2 of BCNU over a 7-day period because of our initial concern about acute toxicity. Since this was not observed, the subsequent patients received BCNU over a 2-day period. Patient 4 also received hydroxyurea (1 g/m^2) for 7 days. Because it was felt that this would complicate the analysis of toxicity and therapeutic efficacy of BCNU, hydroxyurea therapy was not repeated. Marrow was transplanted late in the first four patients (Days 10, 11, 13, and 14) because of the fear of toxicity to the infused marrow by BCNU breakdown products. From experience gained with other transplantation programs, which will be discussed later, marrow was infused in the last five patients on Day 5 or 6 (*see* table 5).

Response

There was only one true partial response among the nine patients. This patient (No. 2) had a > 50% reduction in the size of both inguinal nodes and in the liver size, and a > 50% improvement in liver scan abnormalities. One patient with intracranial relapse of oat cell carcinoma of the lung had an almost partial response by Day 17

TABLE 1.—Patient characteristics and therapy

Patient No. (age/sex)	Diagnosis	Previous therapy*	Extent of disease	Therapy after marrow aspiration and before BCNU therapy*	BCNU therapy	Response†
1 (40/F)	Melanoma	DTIC, BCG; DTIC, ACT-D, *Corynebacterium parvum* (15)	Subcutaneous, pulmonary	—	300 mg/m², Days 1 and 7	< PR (6)
2 (60/M)	Melanoma	DTIC, ACT-D, *C. parvum* (4)	Nodal, liver	—	300 mg/m², Days 1 and 7	PR (6)
3 (60/M)	Melanoma	DTIC, BCG (10)	Nodal, pulmonary, bladder, kidney, subcutaneous, retroperitoneal	—	300 mg/m², Days 1 and 2	Progression
4 (66/M)	Melanoma	DTIC, ACT-D, *C. parvum* (4)	Subcutaneous	—	300 mg/m², Days 1 and 2 (also received hydroxyurea, 1 g/m²)	Stable disease (6)
5 (58/F)	Oat cell carcinoma	VCR, IFOS, VP-16-213, ADR, *C. parvum*, MTX; cranial XRT (11)	Brain	VCR, IFOS, VP-16-213, MTX (1)	300 mg/m², Days 1 and 2	Stable disease (4)
6‡ (53/F)	Oat cell carcinoma	VCR, ADR, CYC, XRT (9)	Brain	—	300 mg/m², Days 1 and 2	< PR (39+)
7 (56/M)	Oat cell carcinoma	VCR, IFOS, VP-16-213, ADR, MTX, *C. parvum*, XRT; CYC, BCNU, VP-16-213§ (23)	Chest	—	300 mg/m², Days 1 and 2	Progression (died on Day 21)
8 (49/M)	Melanoma	DTIC, ACT-D, *C. parvum*; iv MER; *cis*-Pt (11)	Subcutaneous, nodal, liver, adrenal, brain, lung, retroperitoneal, testes	AMSA	375 mg/m², Days 1 and 2	NE (died on Day 16)
9 (49/M)	Melanoma	DTIC, ACT-D, *C. parvum*, *cis*-Pt (8)	Subcutaneous	PALA	375 mg/m², Days 1 and 2	Stable disease (4)

*Nos. in parentheses = duration of therapy in mos. BCG = bacillus Calmette-Guérin; ACT-D = actinomycin D; VCR = vincristine; IFOS = ifosfamide; ADR = Adriamycin; MTX = methotrexate; XRT = irradiation; CYC = cyclophosphamide; iv MER = iv administration of methanol extraction residue of BCG; *cis*-Pt = *cis*-dichlorodiammineplatinum(II); PALA = *N*-(phosphonacetyl)-L-aspartate; AMSA = methanesulfonamide, *N*-[4-(9-acridinylamino)-3-methoxyphenyl]-.

†PR = partial response. NE = not evaluable. Nos. in parentheses = duration of response in wks.

‡This patient received cranial irradiation on Day 17.

§High-dose therapy: CYC = 4.5 g/m², BCNU = 300 mg/m², VP-16-213 = 500 mg/m², with marrow infusion.

before receiving cranial irradiation. This patient's response still persists at 39+ weeks with only questionable abnormalities on computerized axial tomography studies. One other patient (No. 1) showed complete disappearance of subcutaneous disease but no obvious change in her pulmonary nodules. All other patients showed no significant response.

Number of Cells Infused

Table 2 shows the total number of cells collected and infused. The number of cells collected ranged from 1.9 to 4.0 $\times$ 10^8/kg of body weight, with a median of 3.1 $\times$ 10^8/kg of body weight. Because of the marrow hypocellularity in a number of these patients, it is likely that these marrow specimens were heavily contaminated with peripheral blood leukocytes. The percent recovery of nucleated cells after freezing and thawing varied between 31% and 84%, with a median of 60%. The number of cells infused ranged from 1.2 to 2.1 $\times$ 10^8/kg of body weight.

TABLE 2.—Characterization of stored marrow cells

Patient No.	Cells $\times$ 10^8/kg of body weight		% recovery
	Frozen	Infused	
1	4.0	1.6	40
2	3.9	1.2	31
3	3.3	2.0	61
4	2.4	1.6	67
5	3.1	2.1	68
6	3.4	1.7	50
7	2.0	1.0	60
8	2.9	1.7	58
9	1.9	1.6	84
Median	3.1	1.6	60
Range	1.9–4.0	1.2–2.1	31–84

Nonhematopoietic Toxicity

Infusion of the bone marrow was associated with a mild elevation of temperature to approximately 38°C in all patients and skin erythema in two. All patients experienced some nausea and vomiting starting 2 hours after drug administration and lasting, at the most, for approximately 24 hours after cessation of chemotherapy.

Two patients died relatively early, one on Day 16 because of intraperitoneal bleeding from massive liver disease and the other on Day 21 from progressive hypoxia from a fulminant acute pulmonary diffuse interstitial process. This last patient was probably the most extensively treated of all of the nine patients. He had received prior chest irradiation and massive chemotherapy. At the time of death he had a large amount of tumor obstructing the right main bronchus. Autopsy, besides revealing entire replacement of the right upper lobe by oat cell carcinoma of the lung, disclosed an acute alveolitis with no obvious infectious cause. A differentiation between idiopathic adult respiratory distress syndrome and BCNU toxicity could not be made. No other significant nonhematopoietic toxicity such as renal, hepatic, or neurologic was observed.

Hematopoietic Toxicity

Table 3 summarizes the incidence, onset, duration, and

TABLE 3.—Hematopoietic toxicity after high-dose BCNU with ABMT

Toxicity	Incidence (No. of patients with toxicity/ No. treated)	Day of onset		Duration (days)		Time to hematopoietic recovery (days)			
						From ABMT		From BCNU	
		Median	Range	Median	Range	Median	Range	Median	Range
Neutrophils $\times$ 10^9/liter									
0.5	3/8*	16	15–32	11	2–13	17	17–21	28	27–34
1.0	6/8*	18	14–23	13	5–15	22	21–28	34	28–39
1.5	7/8*	17	9–23	14	1–29	26	12–30	34	18–40
Wbcs $\times$ 10^9/liter									
1.0	3/8*	16	16–23	7	1–11	17	12–18	27	23–31
Platelets $\times$ 10^9/liter									
20	8/9	17	14–30	11	1–25	19	13–28	31	19–39
50	9/9	16	13–24	18	5–34	25	14–36	37	20–47
100	9/9	13	11–20	31	14–38†	32	24–48	44	30–54

*1 patient is not evaluable because of early death.

†3 patients are not evaluable for duration of hematopoietic suppression: 1 patient died on Day 16; the other 2 patients are not evaluable for the duration of platelet suppression below or recovery to 100 $\times$ 10^9/liter because of death on Day 21 after BCNU (1 patient) or because other therapy was started before platelet recovery (1 patient).

time to recovery of hematopoietic suppression. Because of early death, one patient (No. 8) was not evaluable for the onset of neutropenia or for the duration and recovery of hematopoietic suppression. Two other patients (Nos. 3 and 7) were not fully evaluable for platelet recovery (one received alternative therapy after reaching 75 × 10^9 platelets/liter and the other died before recovery to 100 × 10^9 platelets/liter.

Table 4 shows the onset of hematopoietic suppression. Of eight patients evaluable for neutropenia, only three had a cell count < 0.5 × 10^9/liter and six had a cell count < 1 × 10^9/liter. Similarly, in only three of eight patients did the total wbc count fall to < 1 × 10^9/liter. All patients showed significant thrombocytopenia. The onset of neutropenia (Days 9–23; median, Day 17) and thrombocytopenia (Days 11–20; median, Day 13) was earlier than the characteristic delayed myelosuppression of BCNU, attesting to the biologic significance of these doses.

The duration of neutropenia in those who experienced neutropenia (tables 3 and 5) was approximately 2 weeks but was variable (range, 1–29 days; median, 14 days). This was probably related to the earlier onset of neutropenia in some patients and the different times of transplantation in others. Nevertheless, neutropenia was relatively short-lived in patients 1, 6, 7, and 9 and was nonexistent in patient 2 (table 5). The duration of wbcs < 1.0 × 10^9/liter was short (range, 1–11 days; median, 7 days) in all but patient 4. All patients showed a relatively long duration of thrombocytopenia (range, 14–38 days; median, 31 days).

Table 6 shows the time after transplantation to hematopoietic recovery. These data are also summarized in table 3 and are simultaneously related to time from the first dose of BCNU. Neutrophil and wbc recovery started approximately 2½–3 weeks after transplantation, and platelet recovery started after 2–4 weeks. Neutrophil recovery was complete by approximately 4 weeks but platelet recovery was slower, taking up to 7 weeks.

When recovery is analyzed from the first dose of BCNU (table 3) in the seven patients who showed neutrophil counts of < 1.5 × 10^9/liter, all had recovered by Day 40 (median, Day 34). Platelet recovery to 100 × 10^9/liter after the first dose of BCNU ranged up to 54 days, with a median of 44 days.

The only bleeding episode was in the patient who died of intraperitoneal hemorrhage. This patient had several such previous episodes of increasing severity before the initiation of this program. The only possible infectious problem occurred in the patient who died of pulmonary failure. As already mentioned, no infectious etiology was discovered either before or during autopsy. Neutrophilia was noted, however, at the time of death.

DISCUSSION

The dose of BCNU (600–750 mg/m²) given to these patients exceeds the doses normally administered (≃ 250 mg/m²) and yet was not associated with significant nonhematopoietic toxicity (22,23). The one patient who experienced probable BCNU-induced pulmonary toxicity had received prior chest irradiation, prolonged aggressive chemotherapy, and a prior BCNU dose of 300 mg/m², thus receiving a total dose of 900 mg/m². There have been isolated reports of BCNU-induced pulmonary tox-

TABLE 4.—Onset of hematopoietic suppression after high-dose BCNU

Patient No.	BCNU therapy	Day of onset						
		Neutrophils × 10^9/liter			Total wbcs × 10^9/liter	Platelets × 10^9/liter		
		< 1.5	< 1.0	< 0.5	< 1.0	< 100	< 50	< 20
1	300 mg/m², Days 1 and 7	28	29	32	29	13	16	18
2	300 mg/m², Days 1 and 7	—	—	—	—	20	24	30
3	300 mg/m², Days 1 and 2	12	14	15	16	12	13	14
4	300 mg/m², Days 1 and 2 (also received hydroxyurea, 1 g/m², Days 1–7)	11	14	16	16	11	14	14
5	300 mg/m², Days 1 and 2	9	14	—	—	12	17	21
6	300 mg/m², Days 1 and 2	19	21	—	—	16	19	23
7*	300 mg/m², Days 1 and 2	17	—	—	—	11	15	16
8†	375 mg/m², Days 1 and 2	—	—	—	—	13	15	15
9	375 mg/m², Days 1 and 2	23	23	—	—	16	20	—

*Patient died on Day 21.
†Patient died on Day 16.

icity after prolonged use of total cumulative doses well above 1000 mg/m² (24,25). It is possible that this effect may not be seen in patients without prior irradiation and with total doses restricted to < 1200 mg/m².

It is difficult to determine if ABMT modified the bone marrow toxicity of BCNU in all of our patients. All patients had been previously treated with chemotherapy and four had hypocellular bone marrows at the time of bone marrow storage, factors that would decrease the quality of the frozen bone marrow stem cells. It has been well-demonstrated in animal systems that prior exposure to alkylating agents, despite complete recovery of peripheral blood counts, does result in stem cell depletion. This becomes particularly evident when such marrow is

TABLE 5.—Duration of hematopoietic suppression

Patient No.	BCNU therapy	Day of transplantation*	Duration (days)						
			Neutrophils × 10^9/liter			Total wbcs × 10^9/liter	Platelets × 10^9/liter		
			< 0.5	< 1.0	< 1.5	< 1.0	< 20	< 50	< 100
1	300 mg/m², Days 1 and 7	13	2	6	11	1	14	22	33
2	300 mg/m², Days 1 and 7	14	—	—	—	—	1	15	22
3	300 mg/m², Days 1 and 2	11	13	18	22	7	25	34	35+
4	300 mg/m², Days 1 and 2 (also received hydroxyurea, 1 g/m², Days 1–7)	10	11	25	29	11	14	19	30
5	300 mg/m², Days 1 and 2	6	—	20	26	—	11	20	32
6	300 mg/m², Days 1 and 2	6	—	7	14	—	5	17	38
7†	300 mg/m², Days 1 and 2	6	—	—	1	—	3	5	10+
8‡	375 mg/m², Days 1 and 2	5	NE§	NE	NE	NE	NE	NE	NE
9	375 mg/m², Days 1 and 2	6	—	5	7	—	—	7	14

*Day after 1st dose of BCNU.
†Patient died on Day 21 but wbc count was 13 × 10^9/liter and platelet count was increasing.
‡Patient died on Day 16; not evaluable (*see* text).
§NE = not evaluable.

TABLE 6.—Hematopoietic recovery after BCNU therapy measured from time of ABMT

Patient No.	Day of transplantation*	Day of—						
		Neutrophil recovery (cells × 10^9/liter)			Wbc recovery (cells × 10^9/liter)	Platelet recovery (cells × 10^9/liter)		
		0.5	1.0	1.5	1.0	20	50	100
1	13	21	22	26	18	19	25	33
2	14	—	—	—	—	17	25	28
3	11	17	21	23	12	28	36	36+†
4	10	17	29	30	17	18	23	31
5	6	—	28	29	—	26	31	38
6	6	—	22	27	—	22	30	48
7	6	—	—	12	—	13	14	15+‡
8	5	NE§	NE	NE	NE	NE	NE	NE
9	6	—	22	24	—	—	21	24

*Day after 1st dose of BCNU.
†Patient started new therapy on Day 36 after transplantation before recovery to 100 × 10^9 platelets/liter.
‡Patient died on Day 21 while showing hematopoietic recovery.
§NE = not evaluable.

used in transplantation experiments (26–30). Therefore, the marrow used for transfusion purposes in these patients may have been suboptimal and did not show the full potential of frozen bone marrow to modify myelosuppression.

Bone marrow was also transplanted relatively late in the first four patients, a factor which would delay recovery. Marrow infusion was delayed in these patients because we anticipated that the cytotoxic breakdown products of BCNU might circulate for extended periods of time (31). More recent experience suggests that bone marrow can be transplanted earlier after BCNU therapy (32).

At present we are conducting a study in leukemic patients utilizing total-body irradiation and high-dose chemotherapy. The irradiation doses (750–950 rads) in combination with extremely high doses of alkylating agents are definitely marrow-ablative. Using marrow collected and frozen during remission and infused at the completion of administration of chemotherapy and irradiation, hematopoietic recovery has occurred in practically all patients (16,18). Granulocyte recovery to 0.5, 1.0, and 1.5 $\times$ 10^9/liter occurred on Days 22, 27, and 34 (median) respectively after transplantation. However, platelet recovery is slower; recovery to 20, 50, and 100 $\times$ 10^9/liter occurred on Days 22, 43, and 55 (median) after transplantation. These recoveries are very similar to those reported in this paper. Gorin et al (15), in a group of patients with acute leukemia, used a combination of drugs consisting of 6-thioguanine, cytosine arabinoside, cyclophosphamide, and CCNU (TACC) followed by ABMT. They reported recoveries to 1 $\times$ 10^9 total wbcs/liter, 0.5 $\times$ 10^9 polymorphs/liter, and 50 $\times$ 10^9 platelets/liter on Days 17, 18, and 23 (median) respectively. Appelbaum et al (14), using a similar program (BACT: BCNU, cytosine arabinoside, cyclophosphamide, and 6-thioguanine) but in nonleukemic patients, reported recovery to 1 $\times$ 10^9 total wbcs/liter and 0.5 $\times$ 10^9 polymorphs/liter on Days 13 and 21 (median) respectively after completion of chemotherapy (approximately 36 hours before transplantation). These recoveries may appear to be slightly faster than those reported in this paper; however, there are a number of reasons why high-dose BCNU may produce more prolonged myelosuppression than the combinations used by these authors.

In murine systems, BCNU produces an early reduction of spleen colony-forming units (CFU-S) to 20% of normal, with a delayed 6-week period to full recovery. Cyclophosphamide produces equivalent CFU-S reduction but recovery occurs over several days. Cytosine arabinoside and other S phase-specific drugs have a low level of CFU-C (colony-forming units in culture) toxicity unless CFU-S are in S phase (33).

These findings in murine systems appear to be similar to the hematopoietic suppression observed in man. Higher doses of cyclophosphamide cause lower hematologic counts, but recovery is still rapid and it has been difficult to demonstrate any shortening of hematopoietic suppression following high-dose cyclophosphamide and ABMT (34). Recovery after cytosine arabinoside, 6-mercaptopurine, and 6-thioguanine is similarly rapid. However, an increase in BCNU dose causes an increase in both the degree and duration of myelosuppression (35). Therefore, in the BACT and TACC protocols, BCNU or CCNU may be the agent most responsible for the prolonged myelosuppression that occurred without the use of ABMT (14,15). However, the doses of nitrosoureas used by these investigators were only slightly higher than the doses normally used. It could be possible that the myelosuppressive effects of high-dose BCNU may be of a magnitude intermediate between that of the BACT and TACC protocols and that of the total-body irradiation and high-dose chemotherapy regimen.

The hematopoietic recovery reported in this paper is similar to that expected from spontaneous recovery after normal doses of BCNU (35). We believe these findings are different from those expected with BCNU administered at these doses in patients with prior chemotherapy and compromised bone marrows who are not receiving ABMT. Hematopoietic suppression usually occurred rapidly and was not characteristic of the delayed thrombocytopenia and neutropenia noted at 4–6 weeks with normal doses of BCNU. The degree of myelosuppression was not the same in all patients.

One patient did not have granulocyte counts $< 1.5 \times 10^9$/liter, and five others did not have granulocyte counts $< 0.5 \times 10^9$/liter or total wbcs $< 1 \times 10^9$/liter. Despite this early fall in the wbc count, confirming the marked biologic effects of these doses, no patient had granulocytopenia prolonged beyond Day 40 after BCNU therapy. The slow platelet recovery after ABMT is fully compatible with our extensive experience in acute leukemia. It has been our experience in other protocols with ABMT that recovery is more rapid when the infused marrow has been exposed minimally to chemotherapy. Platelet recovery under these circumstances is much faster. The definite value of ABMT in modifying the hematopoietic toxicity of the nitrosoureas should be proven with a larger number of patients, and possibly by using an experimental design in which the patient is initially treated with BCNU therapy without ABMT and upon recovery is again treated with BCNU with ABMT infusion. This experimental design would require approximately 3 months and was not considered warranted for our initial studies because of the expected short duration of survival in this group of patients. Using such a design in patients with breast cancer, we have shown the ability of ABMT to hasten wbc recovery after high doses of mitomycin C (50 mg/m^2) compared to the time of wbc recovery in the preceding course after lower doses of mitomycin C (20 mg/m^2) without marrow infusion (unpublished data). As in the patients reported in this paper, the time to platelet

recovery was not shortened by the marrow infusion; however, it did occur at the same time as in the courses with lower doses of mitomycin C without marrow infusion.

In three patients in this study there was objective evidence of response (one partial response and two less than partial responses). The number of patients was too small to determine the superiority of higher doses of BCNU over conventional doses. It is possible that such a program may be more effective in untreated patients using bone marrow not previously exposed to chemotherapy and could be given without significant nonhematopoietic toxicity.

REFERENCES

1. Ashwood-Smith MJ. Preservation of mouse bone marrow at –79°C with dimethyl sulfoxide. Nature 190:1204–1205, 1961.
2. Davies AJS, Playfair JH, and Cross AM. Estimation in vivo of the viability of frozen and stored bone marrow. Transplantation 2:261–270, 1964.
3. Cavins JA, Kasakura S, Thomas ED, et al. Recovery of lethally irradiated dogs following infusion of autologous marrow stored at low temperature in dimethylsulfoxide. Blood 20:730–734, 1962.
4. Cavins JA, Scheer SC, Thomas ED, et al. The recovery of lethally irradiated dogs given infusions of autologous leukocytes preserved at –80°C. Blood 23:38–43, 1964.
5. Thomas ED, LeBlond R, Graham T, et al. Marrow infusions in dogs given sublethal or lethal irradiation. Radiat Res 41:113–124, 1970.
6. Buckner CD, Storb R, Dillingham LA, et al. Low temperature preservation of monkey marrow in dimethyl-sulfoxide. Cryobiology 7:136–140, 1970.
7. Thomas ED, Kasakura S, and Cristoffanini AP. Isogeneic marrow infusion following nitrogen mustard in the dog. Proc Soc Exp Biol Med 118:1019–1022, 1965.
8. Lochte HL, Jr, Kasakura S, Karetzky M, et al. Infusion of marrow in the mouse and dog after thio-TEPA. Blood 21:424–428, 1963.
9. Epstein RB, Storb R, Clift RA, et al. Autologous bone marrow grafts in dogs treated with lethal doses of cyclophosphamide. Cancer Res 29:1072–1075, 1969.
10. Clifford P, Clift RA, and Duff JK. Nitrogen-mustard therapy combined with autologous marrow infusion. Lancet 1:687–690, 1961.
11. Pegg DE, Humble JG, and Newton KA. The clinical application of bone marrow grafting. Br J Cancer 16:417–435, 1963.
12. Kurnick NB, Montano A, Gerdes JC, et al. Preliminary observations on treatment of post-irradiation hematopoietic depression in man by infusion of stored autologous bone marrow. Ann Intern Med 49:973–986, 1958.
13. Kurnick NB. Autologous and isologous bone marrow storage and infusion in the treatment of myelosuppression. Transfusion 2:178–187, 1962.
14. Appelbaum FR, Deisseroth AB, Graw RG, Jr, et al. Prolonged complete remission following high dose chemotherapy of Burkitt's lymphoma in relapse. Cancer 41:1059–1063, 1978.
15. Gorin NC, Najman A, David R, et al. High dose combination chemotherapy (TACC) with and without autologous bone marrow transplantation in patients with acute leukemias. Exp Hematol (suppl) 6:8, 1978.
16. Dicke KA, McCredie KB, Stevens EE, et al. Autologous bone marrow transplantation in a case of acute leukemia. Transplant Proc 9:193–195, 1977.
17. Dicke KA, Lotzova E, Spitzer G, et al. Immunobiology of bone marrow transplantation. Semin Hematol 15:263–282, 1978.
18. Dicke KA, McCredie KB, Spitzer G, et al. Autologous bone marrow transplantation in the patients with adult acute leukemia in relapse. Transplantation 26:169–173, 1978.
19. Dicke KA, Spitzer G, Zander A, et al. Autologous bone marrow transplantation in relapsed adult acute leukemia and solid tumors. Transplant Proc. In press.
20. Schaefer UW, Dicke KA, and van Bekkum DW. Recovery of hemapoiesis in lethally irradiated monkeys by frozen allogeneic bone marrow grafts. Rev Eur Etud Clin Biol 17:483–488, 1972.
21. Hart JS, Drewinko B, Freireich EJ, et al. A rapid and reproducible method for estimating bone marrow clot section cellularity. (Abstr 346) *In* Proceedings of the American Society of Hematology, 16th Annual Meeting, 1973, p 152.
22. Anderson T, DeVita VT, and Young RC. BCNU (NSC-409962) in the treatment of advanced Hodgkin's disease: its role in remission induction and maintenance. Cancer Treat Rep 60:761–767, 1976.
23. Lessner HE. BCNU (1,3,bis(β-chloroethyl)-1-nitrosourea): effects on advanced Hodgkin's disease and other neoplasia. Cancer 22:451–456, 1968.
24. Holoye PY, Jenkins DE, and Greenberg SD. Pulmonary toxicity in long-term administration of BCNU. Cancer Treat Rep 60:1691–1694, 1976.
25. Bailey CC, Marsden HB, and Morris Jones PH. Fatal pulmonary fibrosis following 1,3-bis(2-chlorethyl)-1-nitrosourea (BCNU) therapy. Cancer 42:74–76, 1978.
26. Botnick LE, Hannon EC, and Hellman S. Limited proliferation of stem cells surviving alkylating agents. Nature 262:68–70, 1976.
27. ———. Multisystem stem cell failure after apparent recovery from alkylating agents. Cancer Res 38:1942–1947, 1978.
28. Hellman S, and Botnick LE. Stem cell depletion: an explanation of the late effects of cytotoxins. Int J Radiat Oncol Biol Phys 2:181–184, 1977.
29. Hellman S, Botnick LE, Hannon EC, et al. Proliferation capacity of murine hematopoietic stem cells. Proc Natl Acad Sci USA 75:490–494, 1978.
30. Morley A, and Blake J. An animal model of chronic aplastic marrow failure: late marrow failure after busulphan. Blood 44:49–56, 1974.
31. Stevens EE, and Dicke KA. Bone marrow transfusion in mice treated with BCNU. Proc Am Assoc Cancer Res and ASCO 17:57, 1976.
32. Spitzer G, Dicke K, Lanzotti V, et al. High dose BCNU and BCNU combinations with autologous bone marrow transplantation (ABMT) in adult solid tumor. Proc Am Assoc Cancer Res and ASCO 19:394, 1978.
33. Preisler HD, and Henderson ES. Effect of cytosine arabinoside and 1,3-bis(2-chloroethyl)-1-nitrosourea on hematopoietic precursors in the mouse. JNCI 47:971–977, 1971.
34. Buckner CD, Rudolph RH, Fefer A, et al. High dose cyclophosphamide therapy for malignant disease: toxicity, tumor response, and the effects of stored autologous marrow. Cancer 29:357–365, 1972.
35. Wasserman TH. The nitrosoureas: an outline of clinical schedules and toxic effects. Cancer Treat Rep 60:709–711, 1976.

Multiple Myeloma Resistant to Melphalan: Treatment With Cyclophosphamide, Prednisone, and BCNU[1]

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SUMMARY

Eighty-nine patients with multiple myeloma resistant to melphalan were randomized to receive cyclophosphamide plus prednisone (CP) (47 patients) or cyclophosphamide plus BCNU plus prednisone (CBP) (42 patients). No differences were detected in the two groups prior to therapy. Objective responses occurred in three (7%) of the CP patients and in seven (17%) of the CBP patients. About 40% of the patients in each group achieved some response. Toxic reactions consisted mainly of leukopenia and thrombocytopenia. Median survival was not different in the two groups. The median survival time was 31 months among those patients with an objective response and 9.4 months among those without an objective response. The addition of BCNU to CP increased the frequency of objective response, but not significantly. This triple combination (CBP) cannot be recommended.

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Melphalan is well-established in the treatment of multiple myeloma (1–6). However, resistance to melphalan often develops in patients with an initially satisfactory response, and some patients do not respond to melphalan even initially. Cyclophosphamide also has been used with beneficial results in the treatment of myeloma (7–9); patients with myeloma resistant to melphalan have improved when treated with cyclophosphamide (10). We have observed some response to cyclophosphamide in patients resistant to melphalan (11). Evidently, resistance to one alkylating agent does not necessarily mean resistance to a second alkylating agent.

We have demonstrated responses to BCNU in the treatment of multiple myeloma (12 and Cancer and Leukemia Group B, unpublished data).

Significant benefit has been obtained with the nitrosoureas in a number of advanced mouse tumors refractory to treatment with most antineoplastic agents (13). Combinations of BCNU and 5-fluorouracil have shown superiority over single-drug therapy in upper gastrointestinal tract carcinomas (14). The addition of a nitrosourea appeared to be an improvement over use of cyclophosphamide alone in oat cell carcinoma (15). BCNU in combination with cyclophosphamide has been beneficial in non-Hodgkin's lymphoma (16). Furthermore, BCNU inhibits both RNA synthesis and polyadenylation in Ehrlich cells while cyclophosphamide accelerates Hn RNA and mRNA formation but inhibits polyadenylation (17). Together, the different effects of the two agents might produce a beneficial response.

Prednisone and melphalan in combination produce a better response than melphalan alone in good-risk multiple myeloma patients (3). Poor-risk patients with multiple myeloma also respond significantly better to this combination than to melphalan alone (18).

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Multiple myeloma resistant to melphalan has a poor prognosis. The major objective of this study was to determine whether patients resistant to melphalan would respond to a combination of agents which have shown some benefit separately in multiple myeloma—specifically, whether such patients would respond better to a combination of cyclophosphamide and prednisone (CP) or to a combination of cyclophosphamide, BCNU, and prednisone (CBP).

MATERIALS AND METHODS

Criteria

All patients fulfilled the criteria for the diagnosis of multiple myeloma (19). Resistance to melphalan was defined as lack of objective improvement after at least 3 months of adequate therapy or progression of myeloma after an initial response to melphalan. Criteria for progression were: (*a*) an increase of M protein in serum by at least 50% above the previous nadir obtained during the best response to treatment; (*b*) an increase of M protein in urine by at least 50% above the previous nadir obtained during the best response to treatment; (*c*) a definite progression of osteoporosis, or appearance of new lytic lesions or enlargement of lytic lesions by at least 50% over the initial size; (*d*) an increase of plasma cells by at least 50% above the previous nadir during the best response to treatment; (*e*) a decrease of hemoglobin by 2 g/dl below the maximum that was obtained during the best response to treatment; (*f*) an increase of diameter of measurable plasmacytoma by at least 50% or appearance of new extramedullary plasmacytomas; (*g*) an increase of pain level by two grades; and (*h*) a decrease of performance status by two grades. Establishment of relapse required that these criteria be fulfilled by at least one half of the parameters that had been abnormal. Patients were considered to be resistant to melphalan if they failed to show any objective improvement after at least 3 months of chemotherapy.

For acceptance into the study, patients had to have an estimated survival of at least 2 months and no significant active infection. Previous radiation therapy did not disqualify the patient, but irradiation must have been completed at least 6 weeks before acceptance in the study. Previous low-dose corticosteroid treatment did not disqualify the patient, but previous exposure to any of the nitrosoureas or cyclophosphamide was unacceptable. Patients with other disorders characterized by plasmacytosis in the bone marrow—such as metastatic carcinoma, connective-tissue diseases, chronic infections, and cirrhosis—were excluded. Randomization was not done until after recovery from previous drug-induced cytopenia.

The laboratory determinations initially and during the study were the same as previously reported (11).

Evaluation of Response to Therapy

The following definitions and criteria were used for the purposes of evaluation: (*a*) *serum M protein concentration*—a decrease of $\geq$ 50% from the initial value or disappearance of the M protein; (*b*) *urinary M protein excretion*—a decrease of at least 50% from an initial value of $\geq$ 1.0 g/24 hours; and (*c*) a decrease of the maximum area of measured soft tissue masses by at least 50% without the appearance of new lesions.

Indirect responses to therapy included (*a*) *hemoglobin*—an increase of $\geq$ 2 g/dl that persisted for at least 2 weeks without blood transfusion in patients whose initial hemoglobin concentration had been $\leq$ 11 g/dl; (*b*) *hematocrit*—an increase of 5.5% under the same circumstances, the initial value being $\leq$ 35%; (*c*) *proportion of plasma cells in marrow*—a decrease of 50% from the initial value; (*d*) *recalcification* of bone lesions without development of new lesions during the study; (*e*) *decrease of pain* by two grades (grades: 0–4); and (*f*) *improvement of performance status* by two grades (grades: 0 = patient's activities normal, 1 = patient mildly symptomatic but ambulatory, 2 = patient had moderately severe symptoms but was in bed < 50% of waking time, 3 = patient had severe symptoms and was in bed > 50% of waking time, and 4 = patient completely bedridden).

Treatment Regimens

Patients who fulfilled the criteria for admission to the study were assigned randomly to either of two treatment regimens. On Day 1, 600 mg/m^2 of cyclophosphamide was given iv for regimen 1 and 300 mg/m^2 of cyclophosphamide and 100 mg/m^2 of BCNU were both given iv for regimen 2. On Days 1–7, 0.6 mg/kg of prednisone was added daily to both regimens orally in three equally divided doses.

With both regimens, leukocyte and platelet counts were determined 6 weeks after the initial injections and the patient received another course of therapy. The dose of cyclophosphamide (and BCNU, if included) was altered according to the leukocyte and platelet counts (11). If no leukopenia or thrombocytopenia had occurred in a previous course, the dose of cyclophosphamide, or cyclophosphamide and BCNU, was increased by increments.

RESULTS

Nine of the 98 patients entered in the study were disqualified because of unavailability of records (three patients), inadequate information (two), wrong dose of medication (one), peptic ulcer contraindicating prednisone (one), removal from protocol by error (one), and failure to appear for therapy after randomization (one). Of the remaining 89 patients, 47 received CP (regimen 1) and 42 received CBP (regimen 2).

TABLE 1.—Frequency of response to each regimen

Response	No. of patients (%)	
	CP regimen	CBP regimen
Total evaluable patients*	46	41
Objective only	1 (2)	2 (5)
Objective and indirect	2 (4)	5 (12)
Objective, subtotal†	3 (7)	7 (17)
Indirect only	17 (37)	9 (22)
None	26 (56)	25 (61)

*2 patients without M protein in serum or urine could not be evaluated for objective response.
†Difference not significant.

The mean number of initially abnormal parameters per patient was 4.9 in the CP group and 5.0 in the CBP group. Examination of individual parameters in the two groups revealed no significant difference. The median BUN and serum calcium levels were almost identical. The median time from diagnosis of multiple myeloma to entry in the protocol was 18 months for CP and 17 months for CBP. Thus, there were no significant differences between assigned groups before therapy was started.

Seven patients (17%) in the CBP group and three (7%) in the CP group had an objective response. Seventeen patients (37%) in the CP group and nine (22%) in the CBP group had an indirect response (an increase of hemoglobin or hematocrit, a decrease of plasma cells in marrow, an improved performance status, or a decrease of pain) (table 1). The frequency of individual parameter responses is shown in table 2. Hypercalcemia (Ca > 12 mg/dl) was initially present in four patients, but was not included among the indirect responses because these patients responded to hydration, corticosteroids, or other measures rather than to the chemotherapy. About 40% of the patients in each group had some response.

One (12 patients) to 35 (one patient) therapy courses were given. Only one of the 12 patients with a single course of chemotherapy showed any response, and that was minimal. In general, response correlated directly with the number of courses of chemotherapy.

Of the ten patients who achieved objective response, eight had received melphalan and prednisone for > 6 months prior to entering this protocol. One other patient was randomized to CP because of progressive disease 54 days after melphalan and prednisone had been started, and one patient was randomized to CBP after 3 months of melphalan and prednisone without response.

Toxic reactions consisted mainly of leukopenia and thrombocytopenia (table 3). The nadir of the leukocyte count was virtually the same with the two regimens, but the nadir of the platelet count was lower with the CBP regimen (60,000 vs 98,000/mm^3). The interval to the nadir of both the leukocyte and platelet counts was greater with the CBP regimen than with the CP regimen. This probably reflects the delayed hematologic toxicity of BCNU. Severe infections, mainly septicemia, occurred in five CP patients and in two CBP patients. Only two of these seven patients had leukocyte counts of < 2000/mm^3 at the onset of infection. Mild infections occurred in five patients, almost equally divided between the CP and CBP regimens. None of these five patients had a leukocyte count of < 3000/mm^3.

Survival is shown in figures 1 and 2. Median survival did not differ between the two groups (9.6 vs 9.5 months). As might be expected, the objective responders had significantly longer survival than the nonresponders (31.0 vs 9.4 months; $P < 0.02$). The current status is summarized in table 4. All but six patients (12% of the CBP group and 2% of the CP group) have been removed from the study because of death, lack of response to therapy, relapse, or other reasons.

TABLE 2.—Frequency of abnormal parameters and response

Parameter	No. of patients (%)			
	CP regimen		CBP regimen	
	Abnormal	Responding	Abnormal	Responding
Objective response				
Serum protein	41	1 (2)	30	3 (10)
Urine protein	8	1 (13)	10	3 (30)
Tissue mass	1	1 (100)	3	0
Indirect response				
Hemoglobin	43	9 (21)	38	5 (13)
Marrow	42	8 (19)	37	8 (22)
Bone	39	0	35	0
Pain	27	6 (22)	29	9 (31)
Performance status	28	7 (25)	26	7 (27)

TABLE 3.—Leukocyte and platelet count nadirs

	CP regimen	CBP regimen
Leukocyte count		
Nadir × 10^3/mm^3	2.4	2.5
Day of nadir	46	81
Platelet count		
Nadir × 10^3/mm^3	98	60
Day of nadir	51	80

TABLE 4.—Current patient status

Status	No. of patients	
	CP regimen	CBP regimen
Continues in study	1	5
Had progression or relapse	30	20
Died on protocol	11	15
Lost to followup	4	2
Discontinued for other reasons	1	0
Total	47	42

DISCUSSION

The treatment of patients with multiple myeloma resistant to melphalan is difficult and challenging. Although melphalan is effective in at least two thirds of patients, nearly all patients become resistant to the drug and relapse.

The frequency of response to CP in this study is similar to our previous experience. The addition of BCNU to the two-drug combination did increase the frequency of objective response, but the number of patients responding was still disappointing.

The response of plasmacytomas in hamsters to BCNU has been reported (20). Multiple myeloma has also responded to BCNU (12 and Cancer and Leukemia Group B, unpublished data). In mice, BCNU must have a different mechanism of action because a line of L1210 leukemia resistant to BCNU was found to be sensitive to melphalan (13). Perhaps myeloma patients who are truly resistant to melphalan also exhibit resistance or at least partial resistance to BCNU. The dose of BCNU in our patients may have been inadequate, but more could not be given without undue risk of bleeding or infection. The compromised bone marrow of patients with resistant myeloma who have received previous alkylating agents and radiation therapy, as well as extensive infiltration of the marrow, precludes larger doses of chemotherapy.

Although the addition of BCNU to the CP regimen increases the frequency of objective response, this combination cannot be recommended.

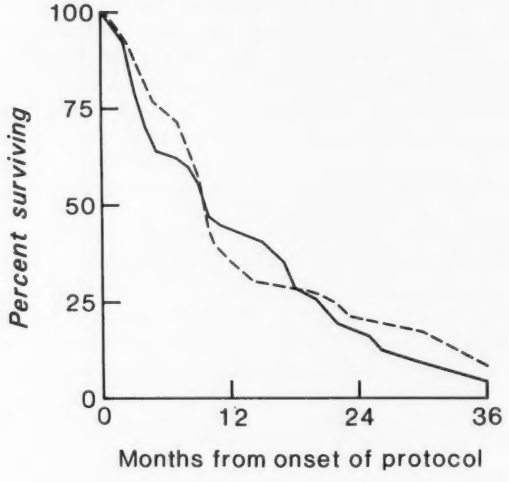

FIGURE 1.—CP (————) and CBP (— —) survival (median: 9.6 and 9.5 mos, respectively; $P = 0.78$).

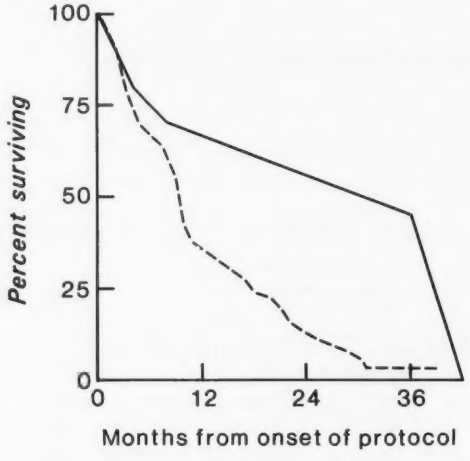

FIGURE 2.—Survival of objective responders (————) and nonresponders (— —) (median: 31 and 9.4 mos, respectively; $P < 0.02$).

REFERENCES

1. Alexanian R, Bonnet J, Gehan E, et al. Combination chemotherapy for multiple myeloma. Cancer 30:382–389, 1972.
2. Bergsagel DE, Sprague CC, Austin C, et al. Evaluation of new chemotherapeutic agents in the treatment of multiple myeloma. IV. L-Phenylalanine mustard (NSC-8806). Cancer Chemother Rep 21:87–99, 1962.
3. Costa G, Engle RL, Jr, Schilling A, et al. Melphalan and prednisone: an effective combination for the treatment of multiple myeloma. Am J Med 54:589–599, 1973.
4. Farhangi M, and Osserman EF. The treatment of multiple myeloma. Semin Hematol 10:149–161, 1973.
5. Hoogstraten B, Sheehe PR, Cuttner J, et al. Melphalan in multiple myeloma. Blood 30:74–83, 1967.
6. McArthur JR, Athens JW, Wintrobe MM, et al. Melphalan and myeloma: experience with a low-dose continuous regimen. Ann Intern Med 72:665–670, 1970.
7. Korst DR, Clifford GO, Fowler WM, et al. Multiple myeloma. II. Analysis of cyclophosphamide therapy in 165 patients. JAMA 189:758–762, 1964.
8. Medical Research Council. Myelomatosis: comparison of melphalan and cyclophosphamide therapy. Br Med J 1:640–641, 1971.
9. Rivers SL, and Patno ME. Cyclophosphamide vs melphalan in treatment of plasma cell myeloma. JAMA 207:1328–1334, 1969.
10. Bergsagel DE, Cowan DH, and Hasselback R. Plasma cell myeloma: response of melphalan-resistant patients to high-dose intermittent cyclophosphamide. Can Med Assoc J 107:851–855, 1972.
11. Kyle RA, Seligman BR, Wallace HJ, Jr, et al. Multiple myeloma resistant to melphalan (NSC-8806) treated with cyclophosphamide (NSC-26271), prednisone (NSC-10023), and chloroquine (NSC-187208). Cancer Chemother Rep 59:557–562, 1975.
12. Salmon SE. Nitrosoureas in multiple myeloma. Cancer Treat Rep 60:789–794, 1976.
13. Schabel FM, Jr. Nitrosoureas: a review of experimental antitumor activity. Cancer Treat Rep 60:665–698, 1976.
14. Moertel CG, Schutt AJ, Reitemeier RJ, et al. Therapy for gastrointestinal cancer with the nitrosoureas alone and in drug combination. Cancer Treat Rep 60:729–732, 1976.
15. Livingston RB. Nitrosourea combinations in lung cancer. Cancer Treat Rep 60:757–760, 1976.
16. Bennett JM, Bakemeier RF, Carbone PP, et al. Clinical trials with BCNU (NSC-409962) in malignant lymphomas by the Eastern Cooperative Oncology Group. Cancer Treat Rep 60:739–745, 1976.
17. Rutman RJ, and Avadhani NG. Distinctive effects of mechlorethamine (NSC-762), cyclophosphamide (NSC-26271), and BCNU (NSC-409962) on transcription in Ehrlich cells. Cancer Treat Rep 60:471–476, 1976.
18. Cuttner J, Wasserman LR, Martz G, et al. The use of low-dose prednisone and melphalan in the treatment of poor-risk patients with multiple myeloma. Med Pediatr Oncol 1:207–216, 1975.
19. Kyle RA, Costa G, Cooper MR, et al. Evaluation of aniline mustard in patients with multiple myeloma. Cancer Res 33:956–960, 1973.
20. Schabel FM, Jr. BCNU [1,3-bis(2-chloroethyl)-1-nitrosourea]: development, antitumor activity, and mechanism of action. *In* Proceedings of the Chemotherapy Conference on the Chemotherapy of Solid Tumors: An Appraisal of 5-Fluorouracil and BCNU (Carter SK, ed). Bethesda, Md, National Cancer Institute, 1970, pp 159–180.

Association Between Steroid Hormone Receptor Status and Disease-Free Interval in Breast Cancer [1]

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SUMMARY

The possibility of an association between steroid hormone receptor status and disease-free interval was examined in 292 patients with breast cancer. Estrogen receptor positivity was associated with a prolonged disease-free interval. This association was independent of age, menopausal status, tumor size, or nodal status. There was no association between the presence or absence of progesterone, androgen, or glucocorticoid receptor and disease-free interval.

[Cancer Treat Rep 63:1271–1277, 1979]

Estrogen receptor-negative tumors have been shown to have high thymidine-labeling indices and therefore more rapid proliferative rates as compared to estrogen receptor-positive tumors (1). Walt et al (2) reported that patients with estrogen receptor-negative tumors have a shorter survival than those with estrogen receptor-positive tumors. Recently, Knight et al (3,4) have shown that patients with estrogen receptor-positive tumors have a prolonged disease-free interval and also a longer survival than patients with estrogen receptor-negative tumors. This report analyzes the association between all four steroid hormone receptors (estrogen, progesterone, androgen, and glucocorticoid) and the disease-free interval as measured from the time of mastectomy until first recurrence.

PATIENTS AND METHODS

Receptor Assays

Biopsy samples of primary tumors taken at the time of mastectomy and metastatic lesions were trimmed of excess fat and nontumorous tissue and divided with a portion submitted for confirming pathology in all cases. Samples for steroid hormone receptor assay were kept on ice and then frozen in liquid nitrogen within 20 minutes. Steroid hormone receptor assays were performed as previously described (5,6). When sufficient sample permitted, Scatchard analyses were performed to quantify the number of binding sites; otherwise, assays were performed in duplicate at one or two concentrations of steroid hormone chosen to exceed several times the expected equilibrium dissociation constant. Data were analyzed using computer-assisted methods (7). A positive estrogen, androgen, or progesterone receptor assay was taken to be $\geq$ 10 femtomols of (^{3}H)-estradiol, (^{3}H)-dihydrotestosterone, or (^{3}H)-R5020 binding per mg of cytoplasmic protein. A positive glucocorticoid receptor assay was taken to be $\geq$ 25 femtomols of (^{3}H)-dexamethasone binding per mg of cytoplasmic protein.

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Patients

From July 1974 to September 1977, 182 women underwent mastectomy for resectable breast cancer. One hundred and ten women had biopsies of metastatic lesions. Patients were from either Georgetown University, Howard University, or George Washington University (Washington, DC), or the National Cancer Institute (Bethesda, Md). Clinical information was obtained from the patient records. Recurrence of disease was documented by liver, bone, and brain scans, chest roentgenogram, and bone survey. Biopsies were usually performed if metastatic lesions were easily obtainable. Steroid hormone receptor status did not influence the frequency or extent of followup examinations. Eighty-three percent of the patients were followed for at least 1 year, 70% for at least 18 months, 42% for at least 2 years, 22% for at least 30 months, and 9% for at least 3 years. The median followup was 23 months.

Statistical Analysis

Comparisons of proportions were performed by the contingency chi-square test with continuity correction. Comparison of continuous or ordered polychotomous distributions was performed by the Wilcoxon rank-sum test adjusted for ties (8). The latter test requires no distributional assumptions of the data, as would a t test. The generalized Wilcoxon test was used to compare distributions of disease-free interval. All significance levels are of the two-sided type.

RESULTS

Figure 1 illustrates the relationship between disease-free interval and estrogen receptor status in 292 patients with breast cancer. In this overall analysis, we included patients who had an assay performed on their primary tumor at the time of mastectomy and also patients who

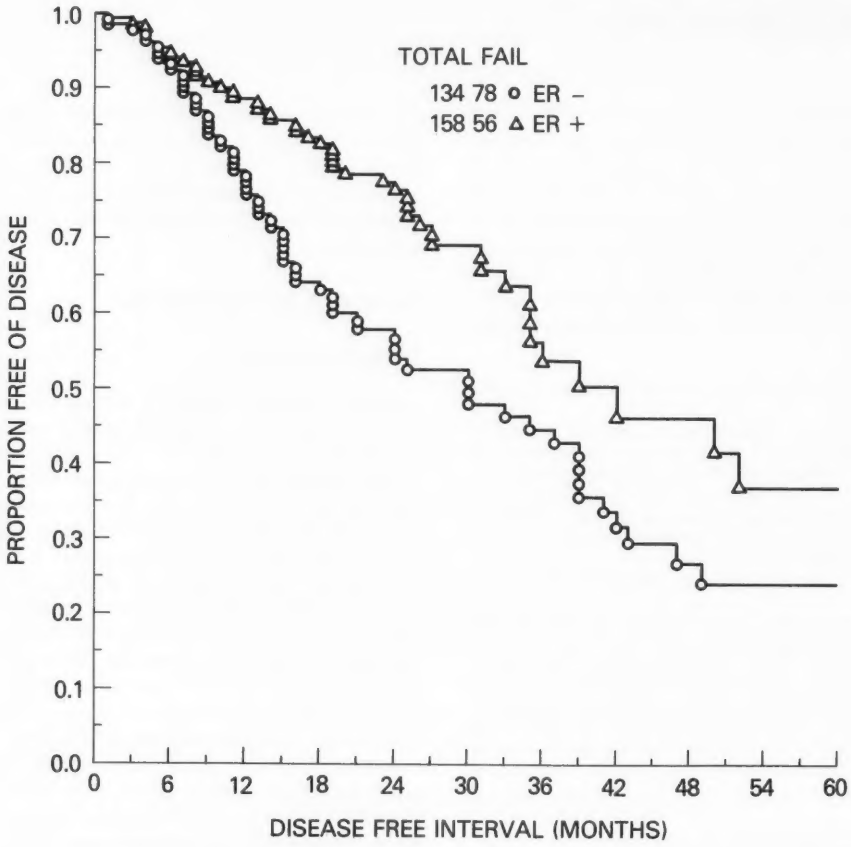

FIGURE 1.—Relationship between disease-free interval and estrogen receptor status in patients with breast cancer.

had an assay performed on metastatic lesions. The inclusion of patients with an assay performed on a metastasis leads to a potential bias in favor of recurrent disease; that is, patients who have not relapsed may not belong to the same receptor population as patients who have relapsed. If metastases tend to be estrogen receptor-negative more commonly than primary lesions, then assays of metastases may suggest (incorrectly) that receptor-negative tumors recur sooner. Even with this potential bias, patients with estrogen receptor-positive tumors have a prolonged disease-free interval when compared to patients whose tumors are estrogen receptor-negative ($P < 0.002$). Similar analysis of disease-free interval as a function of progesterone, androgen, and glucocorticoid receptor status showed no correlation between any of these receptors and the disease-free interval.

Figure 2 shows the relationship between the disease-free interval and the estrogen receptor status in 182 patients with breast cancer who had an assay performed on a sample from their primary tumor obtained at the time of mastectomy. This analysis excludes 110 patients included in figure 1 who had an assay performed on tissue from a metastatic lesion. Of the 182 patients, 79 had estrogen receptor-negative primary tumors and thus far recurrent disease has been documented in 24 patients. Of the 103 patients with estrogen receptor-positive primary tumors, only eight have had recurrent disease. Ninety-one percent of the estrogen receptor-positive patients are free of disease at 2 years compared to 62% of the estrogen receptor-negative patients. The two curves are significantly different at the $P < 0.001$ level.

Table 1 analyzes the estrogen receptor-positive and

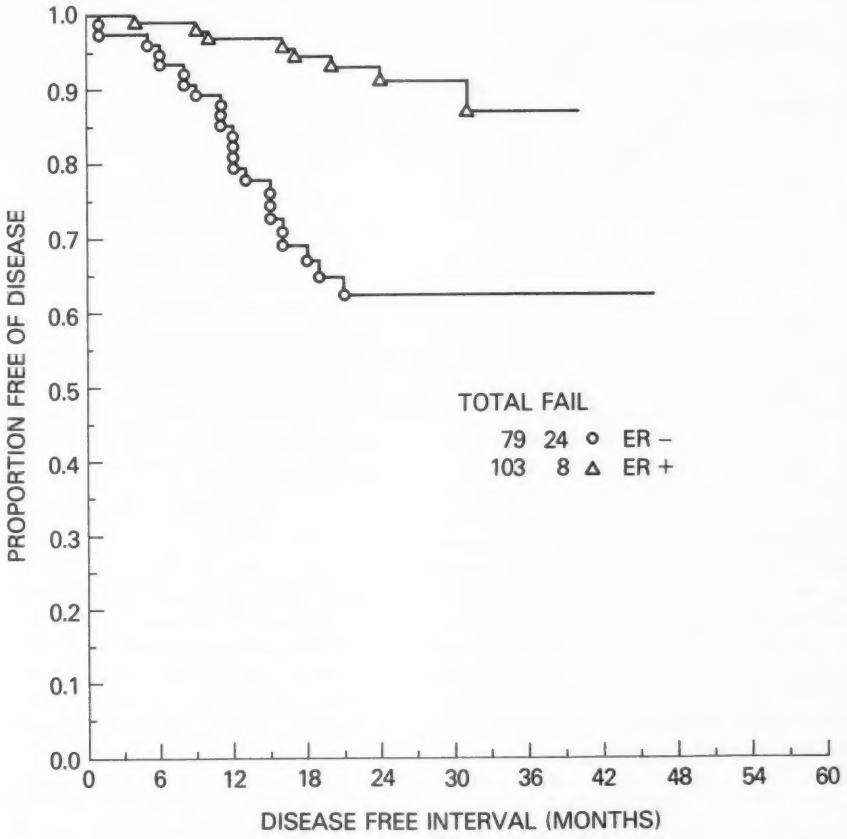

FIGURE 2.—Relationship between disease-free interval and estrogen receptor status in patients with breast cancer. This figure includes only patients who had an assay performed on their primary tumor at the time of mastectomy and excludes patients who had an assay performed on tissues from a metastatic lesion.

-negative groups with regard to age, menopausal status, number of positive nodes, and size of the largest primary nodule. Twenty-five percent of patients in the estrogen receptor-positive group received adjuvant chemotherapy compared to 32% in the estrogen receptor-negative group. In 8% of the patients the adjuvant status was unknown. Although these differences are not statistically significant, we felt it necessary to analyze our data excluding all patients who had received adjuvant therapy. Figure 3 shows the proportion of patients free of disease versus time from primary surgery as a function of estrogen receptor status for the 108 patients who did not receive adjuvant chemotherapy. Forty-one patients were in the estrogen receptor-negative group and 13 have had recurrent disease, compared to five of 67 patients in the estrogen receptor-positive group with recurrent disease ($P < 0.001$). This analysis shows that the influence of estrogen receptor status on disease-free interval is still present in those patients not receiving adjuvant chemotherapy.

Table 2 shows the effect of estrogen receptor status on disease-free interval as a function of other prognostic factors which could affect the disease-free interval. Axillary nodal status, tumor size, and menopausal status are examined. Life-table analyses of these data are graphically illustrated in figures 4A and 4B, 5A, 5B, and 5C, and 6A and 6B. In all categories, estrogen receptor-positive patients have a significantly lower recurrence rate compared to patients whose tumors are estrogen receptor-negative. Thus, it appears unlikely that differences in known prognostic variables can explain the improved disease-free interval in estrogen receptor-positive patients compared to estrogen receptor-negative patients.

Table 3 illustrates the relationship between the dis-

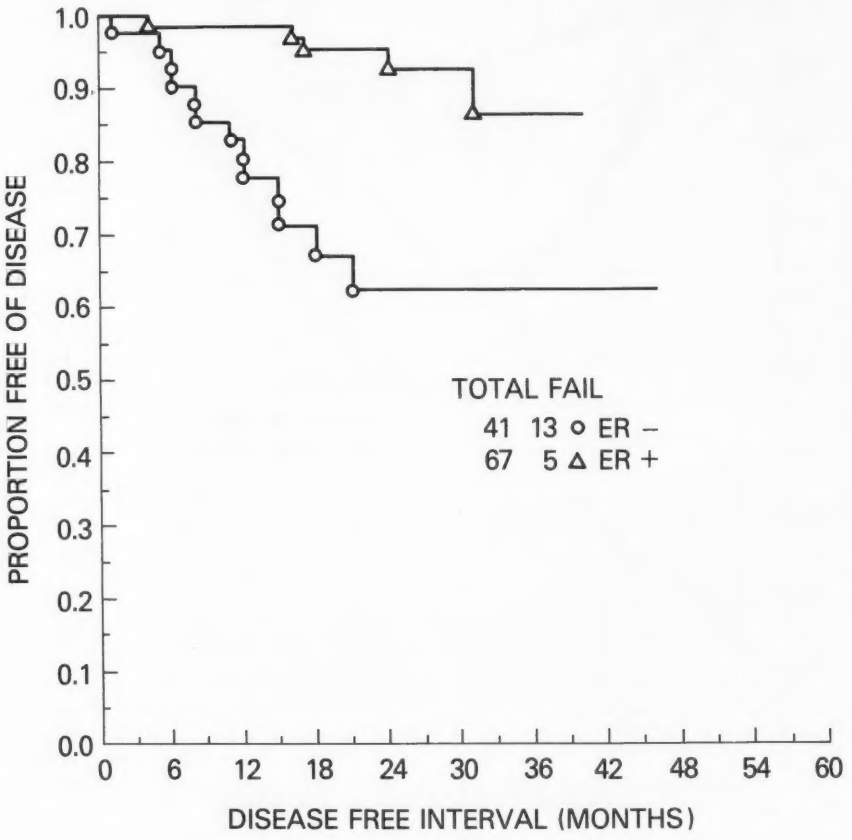

FIGURE 3.—Relationship between disease-free interval and estrogen receptor status in patients with breast cancer. This figure includes only patients who had an assay performed on their primary tumor at the time of mastectomy and who did not receive adjuvant chemotherapy.

TABLE 1.—Comparison of the estrogen receptor-positive and -negative groups with regard to factors that may affect the disease-free interval

Factor	% of patients	
	Estrogen receptor-positive (total No. = 103)	Estrogen receptor-negative (total No. = 79)
Age (yrs)		
< 30	1%	7%
30–39	12%	13%
40–49	13%	26%
50–59	36%	35%
60–69	24%	10%
≥ 70	15%	8%
Menopausal status		
Premenopausal	21%	46%
Postmenopausal	79%	54%
Size of the largest primary nodule		
< 2 cm	47%	52%
> 2 cm	53%	48%
No. of positive axillary nodes		
0	61%	41%
1–3	15%	31%
≥ 4	24%	28%

TABLE 2.—Relationship between estrogen receptor status and disease-free interval as a function of nodal status, tumor size, and menopausal status

	No. of patients with recurrence/total No. of patients	
	Estrogen receptor-positive	Estrogen receptor-negative
No. of positive axillary nodes		
0	5/52	8/30 ($P < 0.02$)
1–3	0/13	6/23 ($P = 0.08$)
≥ 4	2/20	7/21 ($P = 0.05$)
Tumor size		
< 2 cm	4/36	7/30 ($P < 0.04$)
> 2 cm	1/40	10/28 ($P < 0.001$)
Menopausal status		
Premenopausal	1/20	14/32 ($P < 0.01$)
Postmenopausal	4/76	7/37 ($P < 0.01$)

TABLE 3.—Relationship between progesterone, androgen, and glucocorticoid receptor status and disease-free interval in patients with breast cancer

Receptor status	No. of patients with recurrence/total No. of patients	
Progesterone receptor-positive	6/40 (15%)	$P > 0.1$
Progesterone receptor-negative	13/60 (22%)	
Androgen receptor-positive	10/41 (25%)	$P > 0.1$
Androgen receptor-negative	15/78 (19%)	
Glucocorticoid receptor-positive	11/57 (19%)	$P > 0.1$
Glucocorticoid receptor-negative	12/56 (21%)	

ease-free interval and progesterone, androgen, and glucocorticoid receptor status. Although fewer patients had these assays performed on their primary tumors, no relationship could be demonstrated between receptor status and disease-free interval. No differences between the receptor-positive and receptor-negative groups with respect to age, menopausal status, tumor size, or axillary nodal status could be demonstrated which could explain this lack of a relationship.

At the present time our data are too preliminary to analyze the effect of steroid hormone receptor status on patient survival.

DISCUSSION

Recently, Knight et al (3,4) have shown that estrogen receptor positivity is associated with a prolonged disease-free interval and also with increased survival. These authors examined 145 patients. Sixty-three percent had primary tumors which were estrogen receptor-positive and 37% had primary tumors which were estrogen receptor-negative. The estrogen receptor-positive group not only had a prolonged disease-free interval, but in addition this association was independent of age, nodal status, tumor size and location, and postoperative therapy.

Our data agree with these results. Patients with estrogen receptor-positive tumors have a prolonged disease-free interval compared to patients with estrogen receptor-negative tumors. Both groups were similar with respect to age, menopausal status, tumor size, and axillary nodal status. Also, the relationship between estrogen receptor positivity and prolonged disease-free interval was present irrespective of tumor size, nodal status, and menopausal status. In each of these categories, patients with estrogen receptor-positive tumors had a prolonged disease-free interval. Thus, estrogen receptor status appears to be an independent prognostic factor in breast cancer. These data are also in agreement with those of Meyer et al (1) who showed that estrogen receptor-negative tumors have high thymidine-labeling indices and rapid proliferative rates. Both of these factors could lead to the shorter disease-free interval characteristic of estrogen receptor-negative tumors.

Progesterone, androgen, or glucocorticoid receptor status did not appear to influence disease-free interval. Even though the number of patients who had these assays performed on their primary tumors is small, no striking trend exists at the present time. This is a somewhat surprising result, at least with respect to the progester-

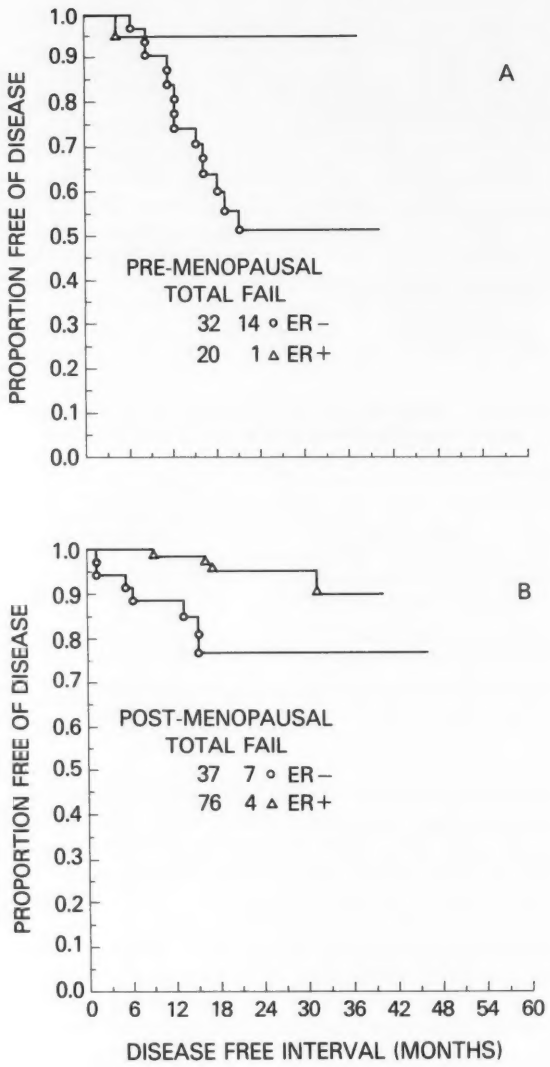

FIGURE 4.—Relationship between disease-free interval and estrogen receptor status in patients with breast cancer. Premenopausal patients are represented in panel A and postmenopausal patients are represented in panel B.

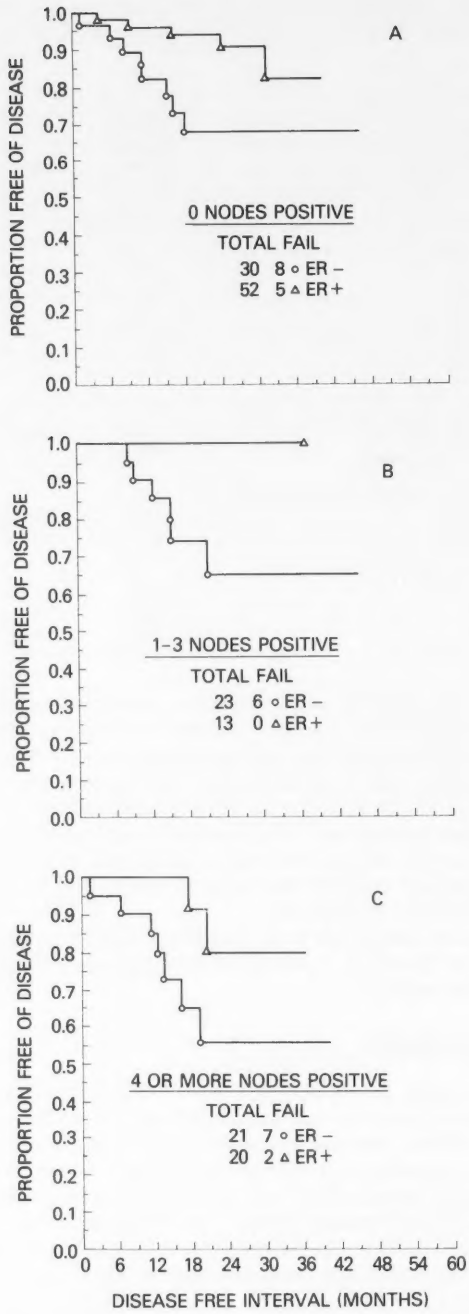

FIGURE 5.—Relationship between disease-free interval and estrogen receptor status in patients with breast cancer. Patients without nodal involvement are represented in panel A, patients with 1–3 positive nodes are represented in panel B, and patients with ≥ 4 positive nodes are represented in panel C.

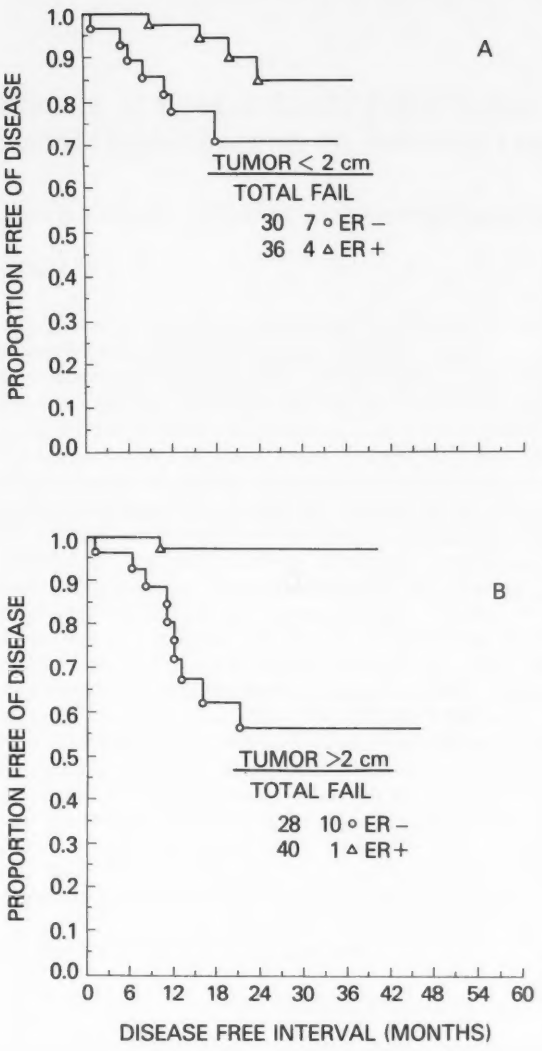

FIGURE 6.—Relationship between disease-free interval and estrogen receptor status in patients with breast cancer. Patients with tumors < 2 cm are represented in panel A and those with tumors > 2 cm are represented in panel B.

one receptor assay. Since there are data which suggest that this receptor is a marker for estrogen responsiveness, one might have predicted that patients with the hormone-dependent estrogen receptor-positive, progesterone receptor-positive tumors would have the longest disease-free interval.

Finally, these data have certain implications for the treatment of breast cancer. First, if estrogen receptor status does indeed influence disease-free interval, then it seems logical to stratify future adjuvant therapy trials according to receptor status as is now done for other prognostic factors such as tumor size and axillary nodal status. Second, caution would be required in comparing disease-free interval in patients receiving adjuvant chemotherapy with disease-free interval in a historical control group, unless estrogen receptor status is known for both groups.

REFERENCES

1. MEYER JS, RAO BR, STEVENS SC, ET AL. Low incidence of estrogen receptor in breast carcinomas with rapid rates of cellular replication. Cancer 40:2290–2298, 1977.
2. WALT AJ, SINGHAKAOWINTA A, BROOKS SC, ET AL. The surgical implications of estrophile protein estimations in carcinoma of the breast. Surgery 80:506–512, 1976.
3. KNIGHT WA III, LIVINGSTON RB, GREGORY EJ, ET AL. Absent estrogen receptor and decreased survival in human breast cancer. Proc Am Assoc Cancer Res and ASCO 19:392, 1978.
4. KNIGHT WA, LIVINGSTON RB, GREGORY EJ, ET AL. Estrogen receptor as an independent prognostic factor for early recurrence in breast cancer. Cancer Res 37:4669–4671, 1977.
5. LIPPMAN ME, and HUFF KK. A demonstration of androgen and estrogen receptors in human breast cancer using a new protamine sulfate assay. Cancer 38:868–874, 1976.
6. LIPPMAN ME, HUFF KK, and BOLAN G. Progesterone and glucocorticoid interactions with receptors in breast cancer cells in long term culture. Ann NY Acad Sci 286:101–115, 1977.
7. AITKEN S, and LIPPMAN ME. A simple computer program for quantification and Scatchard analysis of steroid receptor proteins. J Steroid Biochem 8:77–94, 1977.
8. LEHMAN EH. Non-Parametrics: Statistical Methods Based on Ranks. San Francisco, Calif, Holden-Day Inc, 1975, p 208.

Effects of Systemically Administered Bleomycin or Adriamycin With Local Hyperthermia on Primary Tumor and Lung Metastases [1,2,3]

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SUMMARY

Exposure of cells in tissue culture to bleomycin or Adriamycin during 43°C hyperthermia increased cytotoxicity dramatically compared to exposure at 37°C. This study was designed to test whether this interaction was useful in tumor-bearing animals. C3H mice bearing the KHT tumor were treated with bleomycin (7 or 15 mg/kg) or with Adriamycin (2.5 or 5 mg/kg) with or without local heating of the tumor to 43°C for 30 minutes by 13.56 MHz radiofrequency fields. The effects were assessed by growth delay (mean tumor diameter doubling time) and cure rate. In separate experiments, BALB/c mice bearing EMT6 tumors were treated identically, but tumors were excised 2 hours after treatment and tumor cell survival was assayed by colony formation. Antitumor effects of systemic bleomycin were potentiated by local hyperthermia. The two modalities had to be administered close together in time to observe the potentiation, suggesting a true interaction. There was a "threshold" for bleomycin potentiation in vivo between 42°C and 43°C, just as observed in tissue culture experiments. The antitumor activity of Adriamycin was not potentiated in vivo in these tumor systems except in cell survival experiments at doses higher than those compatible with survival of the host. The toxicity of drug combined with heat was greater than that of either modality alone. Hyperthermia did not adversely affect the incidence or severity of spontaneous lung metastases from KHT tumors. In fact, groups treated with heat and bleomycin had less severe lung metastases than groups treated with bleomycin alone. We conclude that local heating of tumors may be a useful adjunct to systemic bleomycin therapy. In vivo potentiation of Adriamycin by heat, however, could not be demonstrated in these tumor systems.

[Cancer Treat Rep 63:1279–1290, 1979]

Exposure of cells in tissue culture to some chemotherapeutic agents at elevated temperatures in the range of 42°C–43°C increases cytotoxicity dramatically over that seen at 37°C (1,2). This potentiation of cytotoxic effects has been shown for a variety of chemotherapeutic agents including bleomycin (3), Adriamycin (4), and some alkylating agents, including thioTEPA, mechlorethamine, and the nitrosoureas (1,5,6). Relatively few studies have attempted to quantitate this interaction in tumor-bearing animals (2).

There are several reasons why results from studies on combinations of hyperthermia and chemotherapy in vivo might differ from those in vitro. First, pharmacokinetics of drugs administered systemically to animals differ from

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[5]The radiofrequency heating equipment was designed and built by Stavros Prionas. We thank Carolyn Sanders for her expert technical assistance.

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those in tissue culture. Drug levels in tissue culture are likely to remain relatively stable during the experimental period (often ≤ 1 hour) and drug doses are easier to quantitate. Drug doses to which cells in tumors in vivo are exposed are more difficult to specify and are a function of distribution, metabolism, and excretion of the drug by the animals. Hyperthermia itself affects the distribution, metabolism, and excretion of drugs and thus could markedly affect tissue drug levels (7,8). Local hyperthermia can also affect tumor blood flow and local drug transport and metabolism. If circulation to a tumor is significantly changed by hyperthermia, then conditions such as pH and oxygen tension under which tumor cells are exposed to drug may change.[6] Both of these factors have been shown to alter the sensitivity of cells to hyperthermia (6,9). The possible influence on metastatic spread of local hyperthermia to a primary tumor is a clinically important and controversial issue (10,11). Studies of the effects of combined hyperthermia and drugs on cells in tissue culture fail to give vital information on effects on metastases. Finally, effects on normal tissues can be studied in animals.

For these reasons, it seems imperative that the interaction of hyperthermia with chemotherapeutic agents be studied in vivo as well as in vitro to aid in the design of appropriate clinical trials. This study was designed to test under what conditions systemic chemotherapy for EMT6 or KHT solid tumors in mice could be enhanced by local heating of the primary tumor and what effect, if any, the local heating had on spontaneous KHT lung metastases.

MATERIALS AND METHODS

Animals and Tumors

The EMT6 system has been described previously (12). For the experiments reported here, 10^5 EMT6 cells were implanted intradermally into the flanks of female BALB/cKa mice, 3–4 months of age, and the tumors were treated 8–9 days later when the mean tumor diameter (MTD) (the geometric mean of three orthogonal caliper measurements) was approximately 5 mm.

Previous studies showed that EMT6 tumors were often cured by heat alone and, therefore, were not appropriate for a study of the combined effects of heat and drug on tumor growth and cure (13). The KHT mammary carcinoma, a radiation-resistant tumor of low immunogenicity, was chosen for tumor growth studies (14). Viable tumor cells (10^5) were implanted intradermally into the flanks of male C3H mice, 10–14 weeks old, and the tumors were treated 11–12 days later when the MTD was 5–6 mm (the longest diameter was < 10 mm). Untreated KHT flank tumors metastasized spontaneously to lungs in almost 100% of the host mice.

Drug Treatment

Initial drug doses were the highest doses which produced no deaths in groups of tumor-bearing C3H mice given three injections 3 days apart. Bleomycin was administered ip and Adriamycin was administered iv.

Heat Treatment

Flank tumors were heated immediately after drug injection and were brought to treatment temperature within 1 minute. Tumors were then heated by radiofrequency (RF) electromagnetic fields (fig 1). A function generator (13.56 MHz) and power amplifier impressed an electromagnetic field between two plates ("electrodes") of a parallel-plate condenser. For heating of flank tumors, the electrode configuration was such that the tumor was in contact with an electrode that was 1.2 cm in diameter and the opposite flank was in contact with a larger electrode that was 10 cm in diameter. Contact was made through conducting electrode gel. With this configuration, the electromagnetic field lines were most dense in the tumor and were diffuse through the body. Thus, heating was effectively localized to the tumor (13). Some elevation of temperature of the bowel under the tumor probably occurred. However, rectal and core temperatures remained < 38°C in all cases measured.

Commercially available thermistors embedded in the tips of 24-gauge needles were utilized to measure tumor temperature (Yellow Springs Instrument Co, Inc, Ohio) during all treatments. Leads were shielded and the needles were inserted at right angles to the RF field to minimize interference. Difficulties associated with measuring temperature in the presence of an RF field and the estimated accuracy of this system have been described (13). Temperature fall-off studies indicated that the excess temperature introduced by the needle was < 0.2°C. The direction of error was always toward an over-estimate of true average tumor temperature. Tumor temperature was measured throughout each treatment and was regulated by manually adjusting the output of the amplifier. Maps of the temperature distribution in tumors showed uniform heating within ±0.5°C (13). Heating was begun immediately after drug injection, and all treatments were at 43°C for 30 minutes. A photograph of a C3H mouse being treated is shown in figure 2.

Analysis of Effect on Tumor Growth

Animals were treated in five groups. Untreated controls received drug diluent, were anesthetized with Dia-

[6]Fajardo LF, Egbert B, Marmor JB, et al. Effects of hyperthermia in a malignant tumor. Cancer. In press.

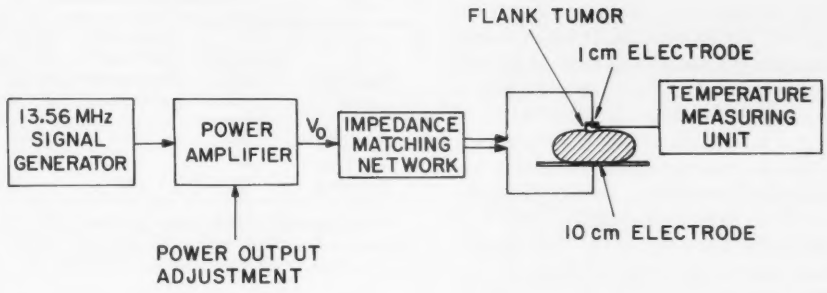

FIGURE 1.—Circuit diagram of RF heating equipment utilized to locally heat flank tumors in mice.

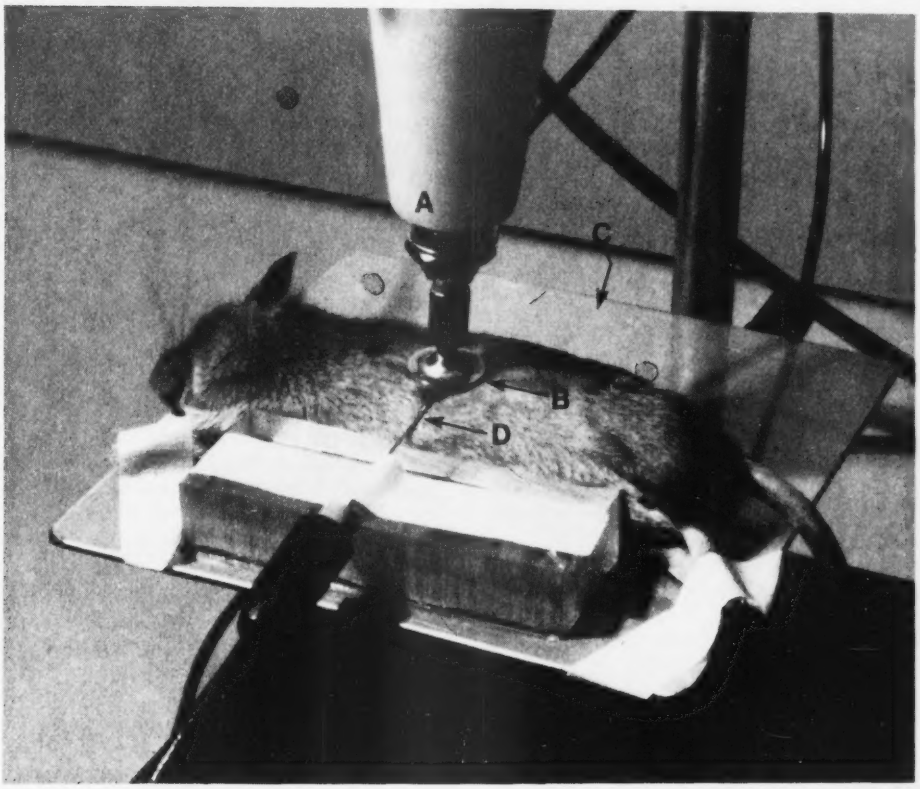

FIGURE 2.—Photograph of a C3H mouse with flank tumor being treated by the RF heating device. A = RF input; B = 1.2-cm diameter electrode; C = 10-cm diameter ground electrode; D = 26-gauge thermistor needle. The flank tumor lies directly under the small electrode; the mouse lies on the large ground electrode.

butal (50 mg/kg), and were sham heated. Heat-alone controls were tumor-bearing mice injected with drug diluent and heated at 43°C for 30 minutes. Drug-alone controls were administered the same drug dose as animals receiving combination therapy and were sham heated. A fourth control group received drug 24 hours before or after heat treatment. This was done to see if simultaneous administration of the two treatments was more effective than addition of individual treatments. Finally, the drug-and-heat group received drug immediately followed by 30 minutes of hyperthermia at 43°C. Serial three-dimensional caliper measurements were made following treatment and the MTD was calculated. The time to double the MTD was used as the criterion for growth delay and this value was compared among groups receiving different therapies. Animals were labeled "cured" if there was complete disappearance of palpable tumor and regrowth of normal hair over the tumor site for a period of > 45 days.

Cell Survival Assay

Methods of cloning tumor cells from the EMT6 sarcoma grown in vivo have been reported previously (11,15). Animals were treated and 2 hours later tumors were excised and minced, and a single-cell suspension was obtained. Appropriate dilutions were plated in Petri dishes. Results were expressed as total clonogenic cells recovered per tumor.

Lung Metastases

C3H mice whose KHT tumors regrew and who survived were killed 23 days after treatment and the lungs fixed in Bouin's solution. Lungs were examined under a dissecting microscope for presence of macroscopic metastases. Lungs were graded for presence and severity of metastases as follows: no macroscopic metastases, 0; four or fewer visible colonies, +1; four to 25 colonies, +2; more than a countable number of colonies or tumors verging on confluent, +3; and lung replaced by tumor and hemorrhage, +4. The average severity of metastases was calculated for each treatment group by calculating the mean grade for the group. Tumor-bearing control animals died 25–35 days after sham treatment due to lung metastases.

RESULTS

Bleomycin

Cytotoxicity.—Figure 3 shows the results of cell survival studies done on cells from EMT6 tumors excised 2 hours after treatment with combinations of bleomycin and heat. The cytotoxicity of bleomycin at normal body temperatures was similar to that described previously (16). Likewise, the degree of cell-killing 2 hours after exposure to heat alone was similar to that previously described.[7] The combination of local hyperthermia (43°C for 30 minutes) given immediately after bleomycin administration resulted in significantly greater cytotoxicity than that seen with either modality alone. Cell-killing due to the combination was more than tenfold greater than that expected from additive effects of the two modalities at a dose of 7 mg/kg and was more than 100-fold greater at a dose of 15 mg/kg. The effect of temperature on this interaction was also examined (fig 4). Little potentiation was noted at temperatures < 43°C. Cell survival after 41°C hyperthermia and bleomycin combined was within the expected additive range. At 42°C, survival was lower than that expected from additive effects but much less dramatically than at 43°C. Ratios of "observed" to "expected" survivals for the three temperatures are shown in table 1. There is a marked increase in this ratio between 42°C and 43°C.

TABLE 1.—Ratio of expected/observed survival values for bleomycin with heat

Bleomycin dose	Temperature (°C)	Expected survival* (clonogenic cells/tumor)	Observed survival (clonogenic cells/tumor)	Ratio
15 mg/kg	41	1700	500	3.4
15 mg/kg	42	1500	300	5.0
15 mg/kg	43	90	0.9	100.0

*The expected value is the product of cell survival for heat alone and drug alone and is the theoretic "additive" effect of the 2 modalities. Values are derived from the data in fig 4.

Growth delay and tumor cure.—Table 2 and figures 5 and 6 show the effect of combined bleomycin and 43°C hyperthermia on KHT tumor cure and growth delay. Cures were seen with combined bleomycin and hyperthermia given together although no cures were seen when the two modalities were given 24 hours apart. Figure 5 shows the median growth delays observed for different combinations of heat and bleomycin. Bleomycin (15 mg/kg) and hyperthermia given together caused a significantly greater growth delay than either modality alone *or* both heat and bleomycin given 24 hours apart. Thus, the two modalities had to be given close together in time in order to show potentiation. The scatter diagram (fig 6) shows that there was little or no overlap between individual tumors treated with combined modality compared to tumors treated with heat or drug alone or both 24 hours apart. The combination of bleomycin and heat was more toxic than either modality alone. Bleomycin (15 mg/kg) could be given five times to C3H mice without

[7] *See* footnote 6.

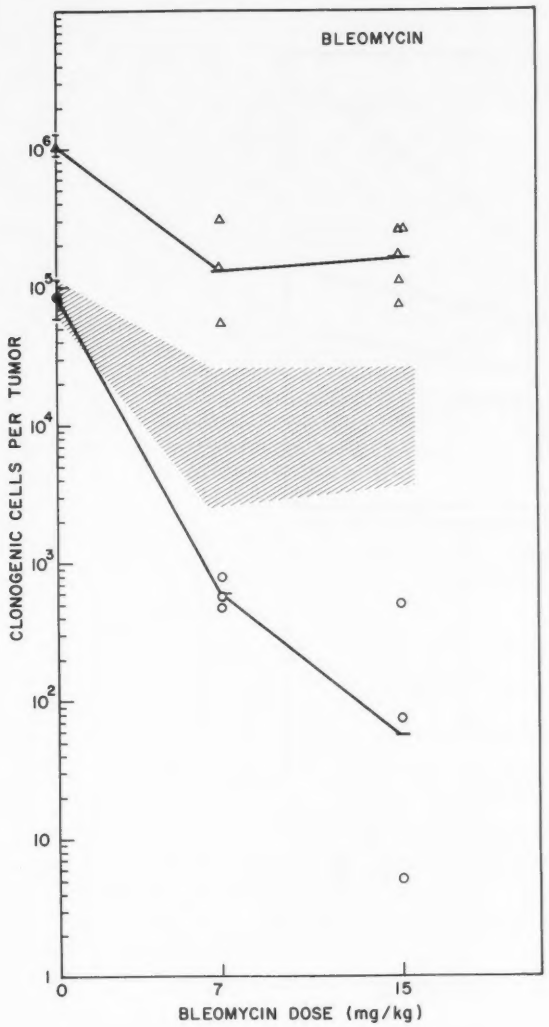

FIGURE 3.—Survival of cells from EMT6 tumors treated in situ with combinations of bleomycin and 43°C hyperthermia. EMT6 tumor-bearing mice were treated with graded doses of bleomycin iv and tumors were either immediately heated to 43°C for 30 mins or sham heated. Tumors were excised 2 hrs after drug administration and cell survival was assayed by cloning. ▲ = 37°C (control); ● = 43°C for 30 mins (no drug); △ = bleomycin without heat; ○ = bleomycin + 43°C for 30 mins. The shaded area is the range of expected "additive" effects and is based on the products of the upper and lower limits of survival after drug and heat alone. The 43°C point is the mean ± SEM of 26 experimental points; the untreated control point is the mean ± SEM of 12 experimental points. Heat + drug points = individual experiments based on pooling 2 treated tumors for analysis and the lines are drawn to the geometric means of these points.

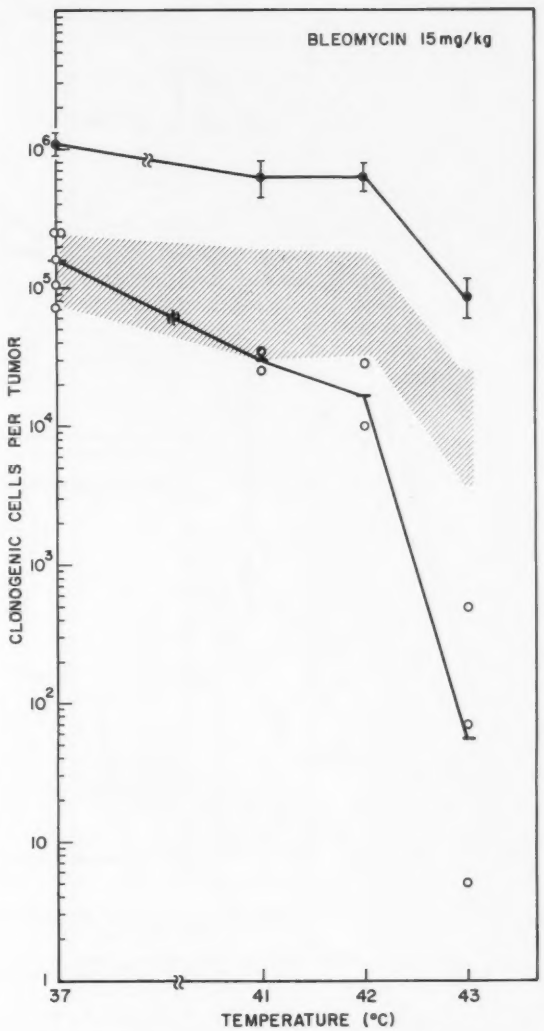

FIGURE 4.—Effect of temperature on survival of cells from EMT6 tumors treated in situ with systemic bleomycin. Procedures were as described in fig 3 except that heating was at 41°C, 42°C, or 43°C for 30 mins. ● = no drug (heat-alone controls); these points = the mean ± SEM of 6–26 experimental points. ○ = bleomycin (15 mg/kg ip); these points = individual experiments with 2 tumors/group. The shaded area is the range of expected "additive" effects for the 2 modalities and was calculated as described in fig 3.

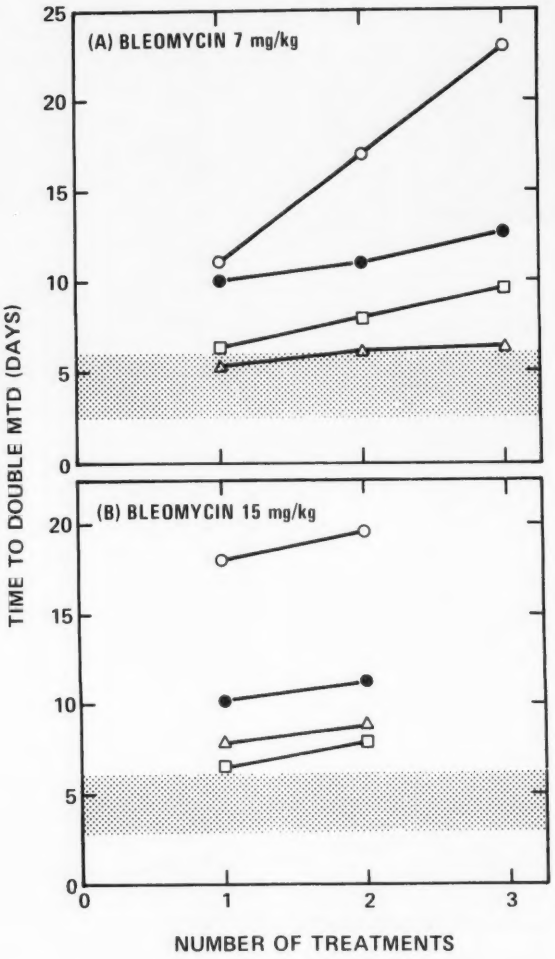

FIGURE 5.—MTD doubling times for KHT tumors in C3H mice treated with combinations of heat and bleomycin. Points shown are the medians for 6–15 animals. The shaded area is the control range (mean ± 2 SD). □ = 43°C for 30 mins; △ = bleomycin alone; ● = bleomycin and 43°C given 24 hrs apart; ○ = bleomycin and 43°C given simultaneously.

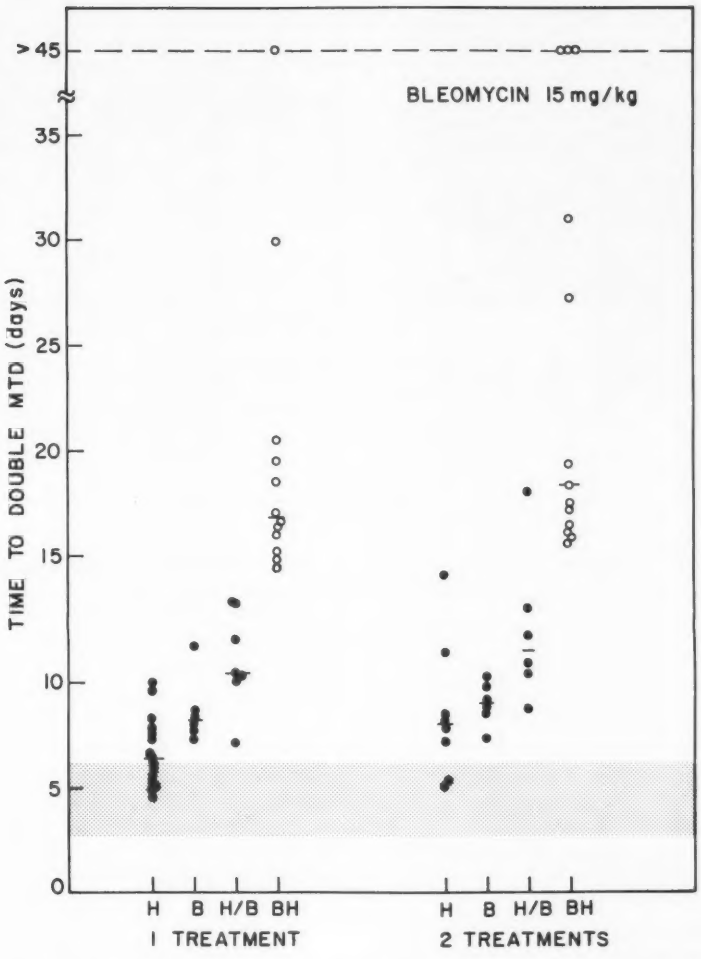

FIGURE 6.—Scatter diagram of MTD doubling times for KHT tumors in C3H mice treated with combinations of heat and bleomycin (15 mg/kg). Each point = 1 tumor in 1 animal. H = heat alone (43°C for 30 mins) and B = bleomycin (15 mg/kg); H/B = heat (43°C for 30 mins) and bleomycin (15 mg/kg) given 24 hrs apart; BH = bleomycin (15 mg/kg) and heat (43°C for 30 mins) given together. The shaded area is the control range (mean ± 2 SD).

TABLE 2.—Incidence of KHT flank tumor cure

Treatment	Incidence of tumor cures/No. of animals treated			
	1 treatment	2 treatments	3 treatments	Total, 1–3 treatments
Control				0/22
43°C for 30 mins	0/15	0/12	0/12	0/39
Bleomycin (7 mg/kg)	0/8	0/8	0/8	0/24
Bleomycin (7 mg/kg + 43°C, 24 hrs apart)	0/8	0/7	0/7	0/22
Bleomycin (7 mg/kg + 43°C, together)	0/7	0/8	0/7	0/22
Bleomycin (15 mg/kg)	0/6	0/6	0/6	0/18
Bleomycin (15 mg/kg + 43°C, 24 hrs apart)	0/7	0/6	—	0/13
Bleomycin (15 mg/kg + 43°C, together)	1/12	3/14	—	4/26
Adriamycin (2.5 mg/kg)	0/12	0/6	0/6	0/24
Adriamycin (2.5 mg/kg + 43°C, 24 hrs apart)	0/6	1/5	—	1/11
Adriamycin (2.5 mg/kg + 43°C, together)	0/6	0/5	—	0/11
Adriamycin (5 mg/kg)	0/6	0/6	0/2	0/14
Adriamycin (5 mg/kg + 43°C, 24 hrs apart)	0/8	0/8	—	0/16
Adriamycin (5 mg/kg + 43°C, together)	0/7	0/11	—	0/18

TABLE 3.—Effect of hyperthermia and drugs on the incidence and severity of spontaneous KHT lung metastases

Treatment	Average grade of lung metastases*				
	1 treatment	2 treatments	3 treatments	Total, 1–3 treatments (± SD)	Incidence of negative lungs/No. of animals treated
Control	—	—	—	3.7 (± 0.5)	0/22
43°C for 30 mins	3.9	2.9	3.5	3.4 (± 0.7)	0/26
Bleomycin (7 mg/kg)	3.5	3.9	3.4	3.6 (± 0.5)	0/21
Bleomycin (7 mg/kg + 43°C, 24 hrs apart)	3.5	3.7	3.0	3.4 (± 0.7)	0/22
Bleomycin (7 mg/kg + 43°C, together)	3.3	1.9	2.7	2.6 (± 1.1)	0/22
Bleomycin (15 mg/kg)	1.7	1.8	—	1.8 (± 1.0)	1/12
Bleomycin (15 mg/kg + 43°C, 24 hrs apart)	2.3	3.2	—	2.8 (± 1.1)	0/12
Bleomycin (15 mg/kg + 43°C, together)	2.1	1.8	—	1.9 (± 1.2)	3/26
Adriamycin (2.5 mg/kg)	3.1	3.2	—	3.1 (± 1.0)	0/16
Adriamycin (2.5 mg/kg + 43°C, 24 hrs apart)	3.3	2.4	—	2.9 (± 1.2)	1/11
Adriamycin (2.5 mg/kg + 43°C, together)	2.3	2.0	—	2.2 (± 1.3)	0/11
Adriamycin (5 mg/kg)	1.3	1.6	—	1.4 (± 0.7)	2/10
Adriamycin (5 mg/kg + 43°C, 24 hrs apart)	†	†	—	1.6 (± 1.0)	2/16
Adriamycin (5 mg/kg + 43°C, together)	†	†	—	2.4 (± 1.3)	2/14

*Lungs were excised 23–25 days after treatment and were examined for macroscopic lung metastases. Each set of lungs was graded on a scale of 0 (no macroscopic metastases) to 4+, depending on severity of metastases (*see* text). Average grade for each treatment group was calculated.

†1- and 2-treatment groups were pooled.

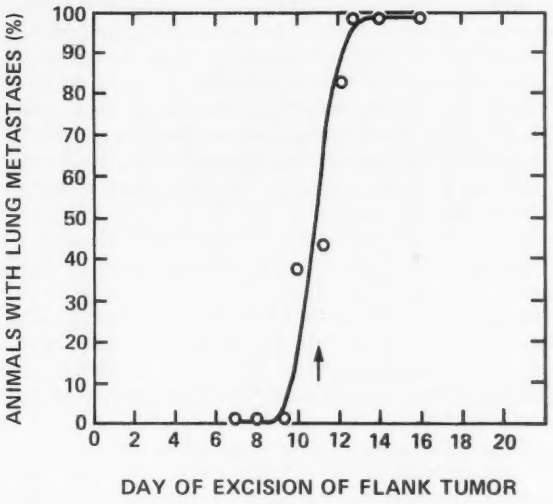

FIGURE 7.—Average day of seeding of lungs from intradermal KHT flank tumors in C3H mice. Flank tumors were excised at varying days after intradermal implantation of 10^5 tumor cells. Lungs were examined for metastases at Day 34 after implant. Arrow = the usual day of flank tumor treatment (Day 11).

toxicity; however, three administrations of this dose of bleomycin together with heat caused five deaths among six animals. Autopsies on animals that died following heat and bleomycin showed edema, necrosis, and perforation of bowel near the tumor. As described previously, this was an area of normal tissue that was probably heated to some extent by the RF field.

Lung metastases.—A series of untreated KHT flank tumor-bearing C3H mice had their flank tumors excised and were then studied for subsequent development of lung metastases (fig 7). These studies showed that metastases first occurred 10–13 days after intradermal inoculation of 10^5 cells. Spontaneous metastases occurred in 100% of mice. Since the usual day of treatment of these tumors was Day 11 (arrow on fig 7), it was possible to assess the effects of local hyperthermia alone as well as in combination with various chemotherapies on the incidence and severity of lung metastases. Lungs were examined 23–25 days after treatment (34–36 days after tumor implantation) and the severity of metastases was graded as previously described. The average grades for each group of control and treated animals are shown in table 3. Heat alone did not appear to either increase or decrease the average severity of metastases compared to controls. When heat was added to bleomycin at a dose of 7 mg/kg, the average severity of metastases was decreased when the two modalities were used together but was not changed when they were used 24 hours apart. Bleomycin at the higher dose (15 mg/kg) was somewhat more effective against lung metastases and the addition

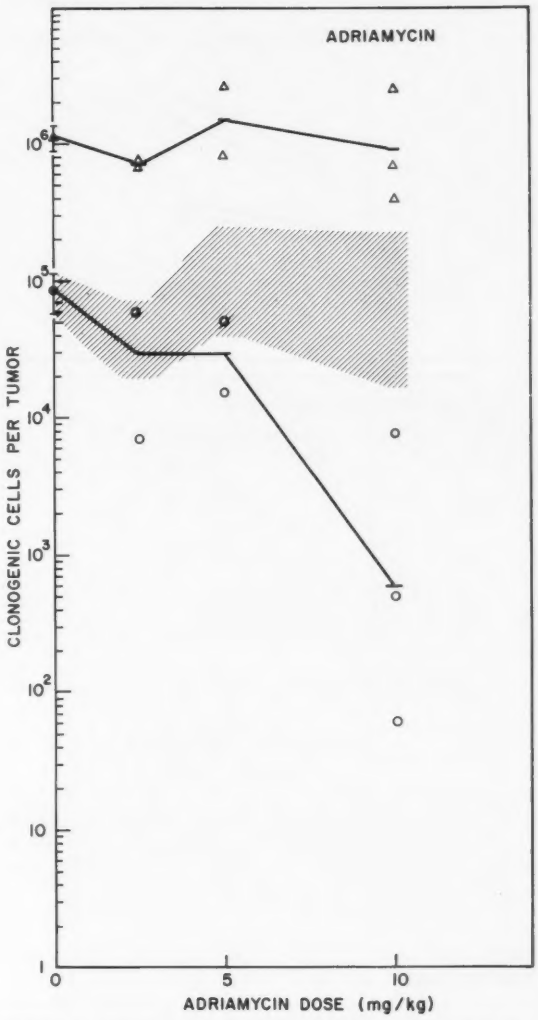

FIGURE 8.—Survival of cells from EMT6 tumors treated in situ with combinations of Adriamycin and 43°C hyperthermia. Experimental procedure was as described in fig 3. ● = 43°C for 30 mins (no drug); ▲ = 37°C (control); △ = Adriamycin without heat; ○ = Adriamycin + 43°C for 30 mins. The shaded area is the range of expected "additive" effects and was calculated as described in fig 3. The 43°C-alone point is the mean ± SEM of 26 experimental points. The untreated control point is the mean ± SEM of 12 experimental points. Other points = individual experiments based on pooling 2 treated tumors for analysis.

of heat did not significantly affect the incidence or severity. One of 12 lungs from animals treated with bleomycin alone (15 mg/kg) was negative for metastases while three of 26 lungs from animals treated with heat and bleomycin were also negative.

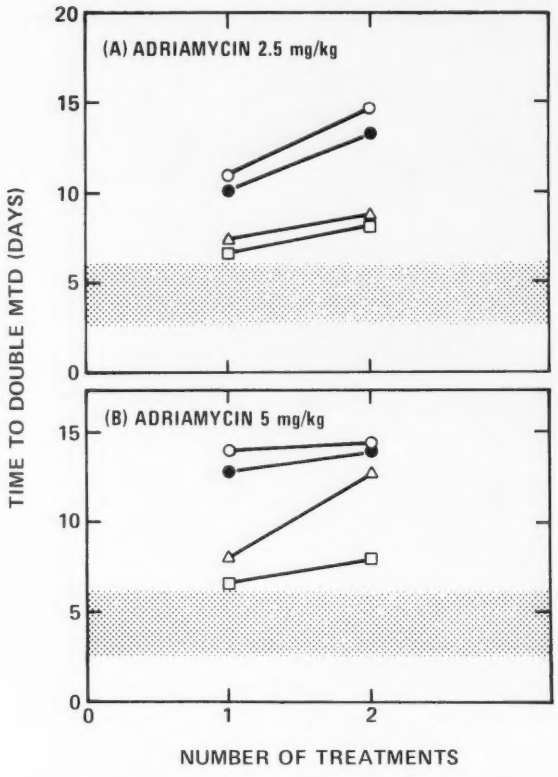

FIGURE 9.—MTD doubling times for KHT tumors in C3H mice treated with combinations of heat and Adriamycin. Points shown are the *medians* for 6–15 animals. The shaded area is the control range (mean ± 2 SD). □ = 43°C for 30 mins; △ = Adriamycin alone; ● = Adriamycin and 43°C given 24 hrs apart; ○ = Adriamycin and 43°C given simultaneously.

Adriamycin

Cytotoxicity.—Figure 8 shows the results of experiments in which EMT6 tumors from mice given systemic Adriamycin, 43°C hyperthermia, or the combination were excised and the cells assayed for survival. The cell survival for all groups was consistent with that previously reported (4). At the highest dose (10 mg/kg), there was enhancement of Adriamycin cytotoxicity by 43°C hyperthermia; however, *no* significant potentiation was seen at doses of 5 or 2.5 mg/kg.

Growth delay and tumor cure.—Adriamycin (10 mg/kg) combined with hyperthermia resulted in 14 deaths among 25 animals 4–7 days after treatment. Thus, this dose was not clinically tolerable and could not be tested for effect on cure rate or tumor growth delay. The effect of lower doses of Adriamycin (2.5 and 5 mg/kg) on tumor cures and growth delay is shown in table 2 and figures 9 and 10. Tumor cures were not observed either with Adriamycin alone or with Adriamycin and hyperthermia. In addition, the combined modality effects did not cause more growth delay than expected from simple additive effects (fig 9). The scatter diagram (fig 10) shows that the groups receiving Adriamycin and heat simultaneously had growth delays similar to those observed in the groups receiving heat and Adriamycin 24 hours apart. There is also considerable overlap between these groups and those given heat or drug alone. As was the case with bleomycin, the combination of Adriamycin and heat was more toxic than either modality alone. For instance, all animals treated three times with Adriamycin (2.5 mg/kg) survived, but 40% of the animals treated three times with this dose of Adriamycin and heat died. Mortality was associated with bowel damage and perforation near the heated site.

Lung metastases.—The average grade and incidence of lung metastases for animals treated with heat and Adriamycin were similar to those for animals treated with Adriamycin alone (table 3).

DISCUSSION

The finding that cytotoxicity of commonly used chemotherapeutic drugs can be markedly potentiated by 42°C–43°C hyperthermia in tissue culture has raised the possibility that local hyperthermia to tumor masses may increase the effectiveness of systemically administered chemotherapeutic agents (1,4). Very few studies, however, have attempted to determine whether this interaction is useful in the unique pharmacokinetic setting of a solid tumor in an animal treated systemically with chemotherapeutic agents. In vitro the combined exposure of cells to 43°C hyperthermia and either bleomycin or Adriamycin substantially potentiates cell-killing (3,4). In our studies, the antitumor effects of systemic bleomycin were significantly increased by local hyperthermia. The antitumor effects of Adriamycin in nonlethal doses could not be demonstrably enhanced by local hyperthermia.

There are at least three possible explanations for the discrepancy between the in vivo and in vitro results for Adriamycin. Sufficiently high concentrations of drug may not be maintained in vivo long enough to show potentiation. The in vitro data indicate that significant potentiation occurs after a 3-hour incubation at a concentration of 0.5 μg/ml (4). While this concentration can be achieved in vivo, the serum half-life of drug is very short (8). Thus, exposure, on the average, will be to a much lower dose in vivo. The drug-dose experiments (fig 8) do suggest that some potentiation of Adriamycin by heat *does* occur in vivo at toxic drug doses. Another possible explanation for the discrepancy between in vivo and in vitro findings for Adriamycin and heat is that timing of the drug and heat dose may not have been optimum in these studies. Heating for too long a period or heating *prior* to drug can

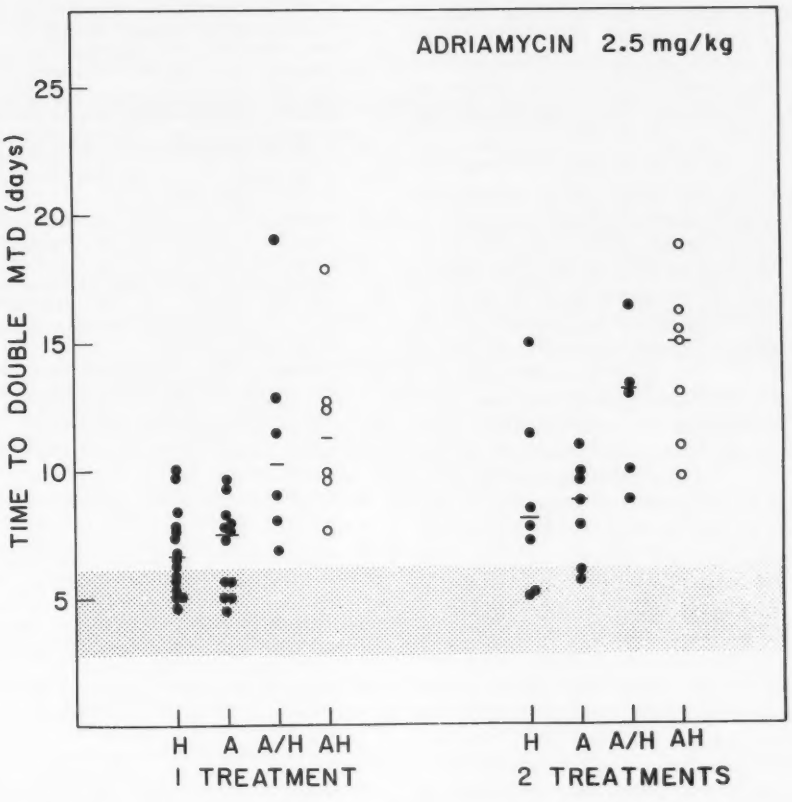

FIGURE 10.—Scatter diagram of MTD doubling times for KHT tumors in C3H mice treated with combinations of heat and Adriamycin. Each point = 1 tumor in 1 animal. H = heat alone (43°C for 30 mins); A = Adriamycin (2.5 mg/kg); A/H = Adriamycin (2.5 mg/kg) and heat (43°C for 30 mins) given 24 hrs apart; AH = Adriamycin (2.5 mg/kg) and heat (43°C for 30 mins) given together. The shaded area is the control range (mean ± 2 SD).

actually make hamster cells in vitro resistant to Adriamycin (17). It is possible that some sensitization could be demonstrated with a different heat fractionation than that applied here. Furthermore, it is also possible that the rate at which thermally induced resistance to Adriamycin develops in mouse cells is different compared to hamster cells. Finally, it should be pointed out that both tumor systems used in these experiments are relatively resistant to Adriamycin. It is possible that different results might be obtained in tumor systems sensitive to Adriamycin.

Clinical observations of effects on tumor growth of the combination of Adriamycin and heat in mammary carcinoma-bearing mice were also reported by Overgaard (18). In his study, Adriamycin was used at a very high dose (25 mg/kg). Eighteen of the 20 Adriamycin-alone controls died from drug toxicity about 7 days after treatment. Heat combined with Adriamycin gave a higher proportion of cures than heat alone. The author suggested that this represented potentiation of Adriamycin by heat. However, it is not possible to tell whether this effect was greater than would be expected from Adriamycin alone or from an additive effect of the two modalities since only two animals treated with Adriamycin alone survived. In any case, it is likely that any discrepancy between Overgaard's study and our study can be explained either by the high dose of Adriamycin used by Overgaard or by differences in tumor sensitivity to Adriamycin.

The results reported here should be helpful in the design of appropriate clinical studies of these agents with hyperthermia. The fact that hyperthermic potentiation of Adriamycin effect can only be demonstrated at systemic doses toxic to the host suggests that this drug may be of little use with systemic hyperthermia or when given systemically alone with local hyperthermia, at least in Adriamycin-resistant tumors. It would be appropriate, however, to study the use of Adriamycin with hyperthermia in *regional perfusion* where higher local concen-

trations of drug for longer periods of time could be achieved without systemic toxicity. The in vivo results for bleomycin also suggest appropriate clinical uses for this drug in combination with hyperthermia. In vivo, as in vitro, there is a temperature "threshold" for potentiation of bleomycin between 42°C and 43°C (table 1) (3). Thus, bleomycin may not be useful when combined with systemic hyperthermia since safe levels of systemic hyperthermia are limited to about 41.8°C (19). A more appropriate use might be with *local* hyperthermia where temperatures in the range of 43°C can safely be utilized. The finding that combined treatment results in increased toxicity also has important clinical implications. Effects of combined treatment on normal tissues will need to be monitored closely.

There has been considerable concern expressed over whether local hyperthermia to a primary tumor might increase the rate of metastasis. Suzuki (20) and Muckle and Dickson (21) noted that combinations of drug and local hyperthermia to a primary tumor gave no survival advantage, despite regression of the primary tumor, because animals died from lung metastases at approximately the same rate as controls. Dickson and Ellis (10), in a later study, suggested that local hyperthermia at 42°C increased the rate of metastasis of the Yoshida tumor in rats. However, this study was not conclusive since the animals treated in this manner had significant *systemic* hyperthermia and 151 of 167 died during treatment or within 24 hours; another ten died 3–12 days later leaving only six survivors for pathologic examination. Yerushalmi (11), using the Lewis lung carcinoma, reported that local heating of a leg primary (42.2°C) slightly delayed the appearance of and decreased the number of lung metastases; whole-body hyperthermia (41.9°C) appeared to slightly accelerate the appearance of lung metastases. In his study, however, animals died from lung metastases at the same time as controls regardless of treatment. Schechter et al (22) also suggested that hyperthermia to the primary tumor may *decrease* the rate of growth of metastatic disease. The studies reported here failed to show any adverse effect of hyperthermia on the incidence or severity of lung metastases. In fact, the combination most effective against the primary tumor (heat and bleomycin) seemed to *decrease* the severity of lung metastases.

REFERENCES

1. Hahn GM. Interactions of drugs and hyperthermia in vitro and in vivo. *In* Cancer Therapy by Hyperthermia and Radiation (Streffer C, ed). Munich, Germany, Urban and Schwartzenberg, 1978, pp 72–79.
2. Har-Kedar I, and Bleehan N. Experimental and clinical aspects of hyperthermia applied to the treatment of cancer. *In* Advances in Radiation Biology (Lett JT, and Adler H, eds). New York, NY, Academic Press, 1976, pp 229–266.
3. Braun J, and Hahn GM. Enhanced cell killing by bleomycin and 43° hyperthermia and the inhibition of recovery from potentially lethal damage. Cancer Res 35:2921–2927, 1975.
4. Hahn GM, Braun J, and Har-Kedar I. Thermochemotherapy: synergism between hyperthermia and Adriamycin or bleomycin in mammalian cell inactivation. Proc Natl Acad Sci USA 72:937–940, 1975.
5. Johnson HA, and Pavelec M. Thermal enhancement of thio-TEPA cytotoxicity. JNCI 50:903–908, 1973.
6. Hahn GM. Metabolic aspects of the role of hyperthermia in mammalian cell inactivation and their possible relevance to cancer treatment. Cancer Res 34:3117–3123, 1974.
7. Ballard BE. Pharmacokinetics and temperature. J Pharm Sci 63:1345–1358, 1974.
8. Mimnaugh EG, Waring RW, Sikic BI, et al. Effect of whole-body hyperthermia on the disposition and metabolism of Adriamycin in rabbits. Cancer Res 38:1420–1425, 1978.
9. Gerwick L, and Rottinger E. Enhancement of mammalian cell sensitivity to hyperthermia by pH alteration. Radiat Res 67:508–511, 1976.
10. Dickson JA, and Ellis HA. Stimulation of tumour cell dissemination by raised temperature in rats with transplanted Yoshida tumours. Nature 248:354–358, 1974.
11. Yerushalmi A. Influence on metastatic spread of whole-body or local tumor hyperthermia. Eur J Cancer 12:455–463, 1976.
12. Rockwell S, and Kallman RF. Cellular radiosensitivity and tumor radiation response in the EMT6 tumor cell system. Radiat Res 53:281–294, 1973.
13. Marmor JB, Hahn N, and Hahn GM. Tumor cure and cell survival after localized radiofrequency heating. Cancer Res 37:879–883, 1977.
14. Kallman RF, Silini G, and van Putten LM. Factors influencing the quantitative estimation of the in vivo survival of cells from solid tumors. JNCI 39:539–549, 1967.
15. Marmor JB, Hilerio FJ, and Hahn GM. Tumor cure and cell survival after localized hyperthermia induced by ultrasound. Cancer Res 39:2166–2171, 1979.
16. Hahn GM, Ray GR, Gordon LF, et al. Response of solid tumor cells exposed to chemotherapeutic agents in vivo: cell survival after 2- and 24-hour exposure. JNCI 50:529–533, 1973.
17. Hahn GM, and Strande DP. Cytotoxic effects of hyperthermia and Adriamycin on Chinese hamster cells. JNCI 57:1063–1067, 1976.
18. Overgaard J. Combined Adriamycin and hyperthermia treatment of a murine mammary carcinoma in vivo. Cancer Res 36:3077–3081, 1976.
19. Pettigrew RT, Galt JM, Ludgate CM, et al. Circulatory and biochemical effects of whole body hyperthermia. Br J Surg 61:727–730, 1974.
20. Suzuki K. Application of heat to cancer chemotherapy. Nagoya J Med Sci 30:1–21, 1967.
21. Muckle DS, and Dickson JA. Hyperthermia (42°C) as an adjuvant to radiotherapy and chemotherapy in the treatment of the allogeneic VX2 carcinoma in the rabbit. Br J Cancer 27:307–315, 1973.
22. Schechter M, Stowe SM, and Moroson H. Effects of hyperthermia on primary and metastatic tumor growth and host immune response in rats. Cancer Res 38:498–502, 1978.

Mechanisms of Sensitivity or Resistance of Murine Tumors to *N*-(Phosphonacetyl)-L-Aspartate (PALA)[1,2]

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SUMMARY

Several biochemical parameters were examined relative to the sensitivity or resistance of representative rodent tumors to *N*-(phosphonacetyl)-L-aspartate (PALA). The activity of the target enzyme, L-aspartate transcarbamylase (ATCase), was evaluated in homogenates of a spectrum of murine neoplasms. ATCase activity was significantly lower in PALA-sensitive as opposed to PALA-refractory tumors. However, among tumors sensitive to PALA, there was no clear-cut relationship between ATCase activity and degree of sensitivity to PALA. Thus, a number of hypotheses were proposed to explain differential sensitivity to PALA in vivo. Enzyme activities in the salvage pathway which phosphorylate pyrimidine nucleosides and deoxynucleosides were found to be greater in refractory tumors. The uptake of PALA, in vitro, though quite slow, was found to be two to eight times greater in two sensitive tumors as compared to the refractory L1210 leukemia. However, in vivo, 24 hours following graduated doses of PALA, nearly identical intratumoral drug concentrations were observed in representative sensitive and refractory tumors. Thus, ultimately, PALA transport would not appear to correlate with differences in drug sensitivity. A number of other biochemical parameters were also found to have no association with sensitivity to PALA in vivo. These included: kinetics of inhibition of ATCase, capacity for restitution of ATCase activity after a dose of PALA, degree of inhibition of ATCase at various doses of PALA, detoxification of PALA by tumor cells, kinetics of uptake of uridine, or catabolism of pyrimidines or pyrimidine nucleosides.

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N-(Phosphonacetyl)-L-aspartate (PALA) is a powerful transition-state analog inhibitor of L-aspartate transcarbamylase (ATCase) from microbial (1) and mammalian (2) cells. By inhibiting ATCase, PALA blocks de novo pyrimidine nucleotide biosynthesis and is cytotoxic to cells in culture; this effect can be reversed by uridine (3,4). PALA exhibits chemotherapeutic activity against a spectrum of solid tumors in rodents, but has little or no activity against transplantable murine leukemias (4–9). The antitumor activity and toxicity of PALA in vivo can be reversed by uridine or carbamyl-L-aspartate, indicative of a specific effect on ATCase (6).

In cultured cells continuously exposed to PALA, emergence of resistant cells was associated with elevation of ATCase activity (10,11). ATCase activity is directly related to proliferation rate in a series of transplantable hepatomas (12) and in normal tissues (13). These two

findings suggest that slow-growing tumors would have lower levels of ATCase and would be more sensitive to PALA than rapidly proliferating tumors. This would explain the unusual spectrum of antitumor activity of PALA in experimental neoplasms. A comparison of cultured cell lines of PALA-sensitive solid tumors and PALA-refractory leukemias showed that naturally occurring resistance to PALA was indeed associated with high ATCase activity (7).

In this report we investigate the relationship of ATCase activity and sensitivity to PALA in a broad spectrum of murine tumors growing in vivo in an attempt to determine whether assays for ATCase in tumor biopsy specimens, rather than in cultured cell lines, might prove useful in predicting tumor response to PALA. It has been suggested (7) that other factors in addition to ATCase activity modulate tumor response to PALA. Thus a number of enzymologic and pharmacologic parameters which could conceivably influence sensitivity to PALA were studied in representative drug-sensitive and drug-refractory tumors. These parameters include: transport of PALA into cells, affinity of ATCase for substrates and PALA, restitution of ATCase following PALA treatment, detoxification of PALA by tumor cells, and uptake and anabolism of pyrimidine nucleosides via the salvage pathway.

MATERIALS

L-[4-^{14}C]-Aspartic acid (specific radioactivity, 19.5 millicuries/mmol), [U-^{14}C]-cytidine (specific radioactivity, 486 millicuries/mmol), deoxy-[2-^{14}C]-uridine (specific radioactivity, 39 millicuries/mmol), [2-^{14}C]-thymidine (specific radioactivity, 60 millicuries/mmol), and [5,6-^{3}H]-uridine (specific radioactivity, 55 curies/mmol) were obtained from Amersham/Searle Corp (Silver Spring, Md). [^{14}C]-PALA (specific radioactivity, 1.51 microcuries/μmol) was a generous gift of Dr. Marjorie Chadwick, Arthur D. Little, Inc (Cambridge, Mass). [^{14}C-Carboxyl]-inulin (specific radioactivity, 2.16 millicuries/g), deoxy-[2-^{14}C]-cytidine (specific radioactivity, 0.05 millicurie/0.38 mg), and [2-^{14}C]-uridine (specific radioactivity, 0.25 millicurie/1.1 mg) were purchased from New England Nuclear Corp (Boston, Mass). The tetrasodium salt of PALA was provided by Dr. Harry Wood of the National Cancer Institute (Bethesda, Md). L-Glutamate oxaloacetate transaminase (EC 2.6.1.1) (190 units/mg of protein; 10 mg/ml) was a product of Boehringer (Mannheim) Corp (New York, NY). Versilube-F50 silicone oil was obtained from the Harwick Chemical Corp (Cambridge, Mass) and had a specific gravity of 1.045 at 25°C and a viscosity of 70 centistokes at 25°C. Dulbecco's phosphate buffered saline and RPMI-1630 medium were from the National Institutes of Health Media Unit (Bethesda, Md). All other reagents and chemicals were of the highest quality available commercially.

METHODS

Therapeutic Studies

For chemotherapy experiments, the ovarian teratocarcinoma or the Ehrlich carcinoma was implanted ip (10^6 cells/mouse) in groups of six to eight female C3H or NIH Swiss mice respectively. Daily ip treatment with PALA was initiated 24 hours later at logarithmically spaced dose levels (including toxic doses). Animals were observed for survival. Presence or absence of a large volume of ascitic fluid in treated groups was determined visually, when all untreated controls showed evidence of florid ascites. For colon carcinoma 38 and M5076 ovarian carcinoma, 2-mm trocar fragments were implanted sc in groups of ten male $B6D2F_1$ mice and ip treatment with PALA on the indicated schedules was initiated 24 hours later. Tumors were measured 3 weeks after implantation and mean tumor weight was estimated by the formula $L \times W^2/2$ (in mm); animals were observed for survival.

Tumors

Tumors were implanted sc or ip in syngeneic hosts. When the growth was well-advanced, but before any deaths were observed, half of the animals bearing a given type of tumor were injected ip with the indicated doses of PALA; the other half received saline. At the appropriate times animals were killed by cervical dislocation. Tumors were homogenized in a medium consisting of 30% DMSO, 5% glycerin, 0.1 M Tris-HCl (pH 8.4), 0.1 M KCl, 0.2 mM EDTA, and 2 mM dithiothreitol, using a glass vessel and motor-driven Teflon pestle. The homogenate was spun at 12,000 × *g* for 3 minutes in an Eppendorf Centrifuge, the pellet of cellular debris was discarded, and the supernatant was stored at –87°C.

L-Aspartate Transcarbamylase

ATCase was assayed in a reaction mixture of 15 μl, containing 5 μl of 10.45 mM L-[4-^{14}C]-aspartic acid (specific radioactivity, 2.39 millicuries/mmol), 5 μl of 12.5 mM carbamyl phosphate in 0.05 M Tris-HCl buffer (pH 8.5) or 5 μl of 0.05 M Tris-HCl buffer (pH 8.5), and 5 μl of supernatant. The reactants were driven together by acceleration of the tubes containing them to 12,000 × *g* for 1 second in the Eppendorf Centrifuge in order to mix, since more conventional means of mixing, ie, flick of the finger or vortex mixing, tend to disperse rather than coalesce the small volumes of reactants used in this assay. The reaction mixtures were incubated at 37°C for 15 minutes and the reaction was terminated by heating at 95°C for 2 minutes. Excess L-[4-^{14}C]-aspartic acid was dissipated by the addition of 50 μl of decarboxylation reagent consisting of 50 μl of 0.66 M sodium acetate buffer, pH 5.0, containing 6 mM α-ketoglutaric acid, 8.5 mM $ZnSO_4$, and

1 IU of GOT; the assemblies were then incubated in a hood, in open vessels, overnight at room temperature. The product, [4-^{14}C]-carbamyl-L-aspartate, which remains after decarboxylation was counted by immersing the entire vessel in vials containing 20 ml of liquid scintillant and counting the radioactivity in a Beckman LS-230 scintillation spectrometer at 83% efficiency. Formation of [4-^{14}C]-carbamyl-L-aspartate was verified by loading a deproteinized reaction mixture onto a 1 × 10-cm column of Hamilton HA-X4 resin, equilibrated with a buffer consisting of 0.025 M lithium, 5.2 mM citrate, and 0.021 M chloride (pH 2.65), and developed at a flow rate of 0.67 ml/minute. [4-^{14}C]-Carbamyl-L-aspartate was eluted at 45 minutes in this system.

Protein determinations were made with the Bio-Rad dye binding reagent (Bio-Rad Laboratories, Richmond, Calif) (14). ATCase activity was expressed as nmols of carbamyl-L-aspartate formed/mg of protein/hour.

Kinetic Studies

For this study, tumor extracts were partially purified by centrifugation of the crude extract for 1 hour at 100,000 × *g* at 4°C; the supernatant from this step was then recentrifuged at 100,000 × *g* for 16 hours at 4°C. The protein pellet from this centrifugation (15-fold purified) was dissolved in the homogenizing buffer to an appropriate concentration. For kinetic studies the reaction mixtures in a total volume of 20 µl contained 5 µl of L-[4-^{14}C]-aspartic acid (final concentration, 0.078–5.0 mM), 5 µl of carbamyl phosphate (final concentration, 0.0325–8.0 mM) in 0.05 M Tris-HCl buffer (pH 8.5) or 5 µl of 0.05 M Tris-HCl buffer (pH 8.5), 5 µl of PALA (final concentration, 0.15–0.6 µM) or 5 µl of water, and 5 µl of appropriate partially purified tumor extract. The reactants were driven together by brief (1 second) centrifugation in an Eppendorf Centrifuge at 12,000 × *g*, incubated at 37°C for 15 minutes, and processed as detailed above.

Estimation of PALA

The concentration of PALA in tumors and tissues was estimated by a radiometric assay based on inhibition of exogenous ATCase (15).

Restitution of L-Aspartate Transcarbamylase Activity

One million cells of Lewis lung carcinoma or L5178Y/AR leukemia were transplanted sc into male BDF_1 mice. Ten days later, tumor-bearing mice were divided into two groups: the first group received an ip injection of saline and served as control; the second group received 400 mg/kg of PALA ip. Ten mice from each group were killed at the appropriate time periods; tumor was removed and homogenized and measurements of ATCase activity and PALA concentrations were carried out as detailed earlier.

Dose Response of L-Aspartate Transcarbamylase to PALA In Vivo

Mice bearing subcutaneous nodules of either L5178Y/AR leukemia or Lewis lung carcinoma were given ip injections of PALA, at the indicated doses, or saline. Twenty-four hours later tumor nodules were removed and ATCase and PALA measurements were carried out according to the methods detailed earlier.

Transport of PALA

Tumor cells for the study of transport of PALA were prepared by the following techniques:

1. One × 10^5 cells of L1210 leukemia were transplanted ip into male BDF_1 mice; 5 days later, tumor cells were aspirated from the peritoneum.
2. One × 10^6 cells of mouse ovarian teratocarcinoma were transplanted ip into female C3H mice; 7 days later, tumor cells were aspirated from the peritoneum.
3. One × 10^6 Ehrlich ascites cells were transplanted ip into male BALB/c mice; 7 days later, tumor cells were aspirated from the peritoneum.

Cells from the above tumors were collected in RPMI-1630 medium containing 10% heat-inactivated fetal calf serum and 25 mM HEPES (pH 7.4). They were washed twice in the same medium and the cell density was adjusted to 1 × 10^7 cells/ml. Transport studies were performed at 37°C with gentle agitation of the cells. [^{14}C]-PALA was added to yield a final concentration of 250 µM, and at appropriate times, 900-µl aliquots were transferred into microcentifuge tubes containing 750 µl of Versilube-F50 silicone oil. Transport was terminated by centrifugation of the cells through the oil at 12,000 × *g* for 1 minute in an Eppendorf Centrifuge. The cell pellets were solubilized in 1.5 ml of 0.2 N NaOH at 37°C for 3 hours. Scintillation fluid was then added and radioactivity was measured by liquid scintillation spectrometry. Data were corrected for entrapped extracellular drug using [^{14}C]-inulin as a marker.

Metabolism of PALA

By microsomes.—Hepatic microsomes were prepared from rat liver (16). In a final volume of 1 ml were admixed 0.1 microcurie (0.3 µmol) of [^{14}C]-PALA, 16 mg of microsomal protein, and 5 µmols of NADPH. Controls received boiled microsomal protein in lieu of the intact organelles. After 1 hour in an atmosphere of O_2 or N_2, protein was denatured by the addition of 50 µl of 2 N HCl and the supernatant was subjected to chromatography on a 1.0 × 10-cm column of Hamilton HA-X4 resin equilibrated with lithium buffer containing 0.025 M lithium, 5.2 mM

citrate, and 0.021 M chloride (pH 2.65) (buffer I) and operated at a flow rate of 0.67 ml/minute. After loading the sample, this column was developed with the equilibrating buffer for 90 minutes at 37°C, followed by buffers at the same pH containing 0.063 M lithium, 13.2 mM citrate, and 0.053 M chloride (buffer II); 0.126 M lithium, 26.4 mM citrate, and 0.106 M chloride (buffer III); 0.253 M lithium, 52.0 mM citrate, and 0.213 M chloride (buffer IV); or 0.380 M lithium, 78.0 mM citrate, and 0.319 M chloride (buffer V), applied for periods of 60 minutes each. PALA was eluted at 215 minutes.

By tumor cells.—L5178Y/AR leukemia and mouse ovarian teratocarcinoma cells were separated from the ascitic fluid of mice bearing the respective tumors. The cells were washed twice with Hanks' balanced salt solution and suspended in the same medium to a cell density of 1–10 $\times$ 10^7 cells/ml. To 1 ml of this cell suspension, 0.1 microcurie (0.3 μmol) of [^{14}C]-PALA was added and incubated at 37°C for 1 hour in an atmosphere of 95% air and 5% CO_2. The cell pellet was denatured by the addition of 50 μl of 2 N HCl and the supernatant was subjected to chromatography as detailed above.

Uridine Uptake Studies

Cells from the respective tumors were aspirated from the peritoneum with Dulbecco's phosphate buffered saline containing 0.1 mM bovine serum albumin and 0.25% glucose (pH 7.4). They were washed twice in the same medium and suspended at a cell density of 2 $\times$ 10^6 cells/ml at 37°C with gentle agitation. Labeled uridine (1.25–40 μM) was added at 0 time and uptake was terminated after 18 seconds by centrifugation of the cells through silicone oil. The cell pellets were processed as described under "Transport of PALA."

Utilization of Pyrimidines

Pyrimidine Nucleoside Kinase Levels in Tumors In Vitro

L1210 and L5178Y/AR leukemia, Ehrlich ascites carcinoma, and mouse ovarian teratocarcinoma cells were collected and washed as detailed under "Transport of PALA." Subcutaneous nodules of Lewis lung carcinoma were procured as detailed under "Tumors." Tumors were homogenized in 0.1 M Tris-HCl (pH 7.6) containing 0.5 mM EDTA and 1.0 mM dithiothreitol. The homogenate was centrifuged at 12,000 $\times$ *g* for 3 minutes, and the supernatant was used as the source of enzyme. Reactions were carried out in 15-μl volumes containing 5 μl of [^{14}C] precursor (0.25 microcurie, 5 nmols), 5 μl of 0.01 M ATP-$MgCl_2$ in 0.05 M Tris-HCl buffer (pH 8.5) or buffer alone, and 5 μl of extract. The mixture was incubated at 37°C for 15 minutes and the reaction was terminated by adding 10 μl of 2 N HCl. The vessels were centrifuged at 12,000 $\times$ *g* for 3 minutes and 5 μl of the sample was subjected to ascending paper chromatography on Whatman 3M paper for 18 hours, using isopropanol:EDTA (saturated solution): toluene:14 M NH_4OH (320:44:40:4) as solvent (17). The phosphorylated products stay at the origin.

To study the catabolism of the pyrimidine ring by L1210 and L5178Y/AR leukemias, ovarian teratocarcinoma, and Ehrlich ascites carcinoma cells, tumors were transplanted as described under "Transport of PALA." Cells were aspirated from the tumor-bearing mice as detailed earlier and washed twice with Dulbecco's phosphate buffered saline containing 2% glucose and 25 mM HEPES. Washed cells were suspended in the same medium. To 1.5 ml of cell suspension (5 $\times$ 10^7 cells/ml) in polypropylene scintillation vials, 1.0 microcurie (11–17 μM) of [2-^{14}C]-uracil, [2-^{14}C]-uridine, or deoxy-[2-^{14}C]-uridine was added and incubated at 37°C for 1 hour in an atmosphere of 95% air and 5% CO_2. The reaction was terminated by adding 100 μl of 2 N HCl and the liberated [^{14}C]-O_2 was collected by closing the vials with caps containing 50 μl of 40% KOH in the center wells (18) and further incubating at 37°C for 2 hours. The caps containing the center wells were removed and screwed onto a glass vial containing 20 ml of scintillation fluid, and the radioactivity was counted in a Beckman scintillation spectrometer.

RESULTS

PALA has been evaluated in a broad spectrum of transplantable murine solid tumors and leukemias and has been found to be active in the former and ineffective in the latter (4–9). The antitumor activity of PALA in four additional mouse solid tumors which were subjected to biochemical evaluation in the current study is shown in table 1. Against two ascitic tumors, Ehrlich carcinoma and ovarian teratocarcinoma, daily treatment with PALA delayed the appearance of ascites and significantly prolonged survival. PALA strongly inhibited growth of sc implanted M5076 ovarian carcinoma, increased lifespan by 61%, and produced three long-term tumor-free survivors among ten animals. Against sc implanted colon carcinoma 38, PALA was less effective, with only 74% inhibition of tumor growth at a maximally tolerated dose.

ATCase activity was measured in homogenates of 13 mouse tumor lines including eight solid tumors of varying degrees of sensitivity to PALA, four PALA-refractory leukemias, and a cytosine arabinoside (ara-C)-resistant subline of P388 leukemia which has proven to be collaterally sensitive to PALA (19). The mean specific activity of ATCase in the nine PALA-sensitive tumors (241 $\pm$ 126 nmols of carbamyl-L-aspartate formed/mg of protein/hour) was significantly ($P < 0.01$) lower than the ATCase activity in the four refractory tumors (526 $\pm$ 99 nmols/mg/hour) (table 2). There is obviously no exact correspond-

TABLE 1.—Activity of PALA in vivo against 4 transplantable carcinomas*

Tumor	Site of implantation	Schedule of PALA administration	Optimal dose (mg/kg/injection)	Tumor growth inhibition	MST† (treated/control)	% ILS‡	Long-term survivors§
Mouse ovarian teratocarcinoma	ip	Days 1–4	280	0/7 with ascites on Day 12¶	30/20	50	1/7
Ehrlich ascites carcinoma	ip	Days 1–21	31	2/6 with ascites on Day 14¶	48.5/22	120	2/6
		Days 1–13	144	0/8 with ascites on Day 11¶	38.5/21	83	0/8
Colon carcinoma 38	sc	Days 1–4	240	74% TWI on Day 21**	60/41	46	0/10
M5076 ovarian carcinoma	sc	Every other day, Days 1–21	80	98% TWI on Day 22**	45/28	61	3/10

*Tumor implantation and PALA treatment were carried out according to the methods detailed in the Methods section.
†Median survival time in days.
‡% increase in lifespan.
§Survivors on Day 75 without evidence of tumor.
¶Ascites grossly evident in all untreated controls on these days.
**% tumor weight inhibition (TWI) relative to untreated controls as determined by caliper measurement of perpendicular diameters.

TABLE 2.—ATCase activity of a spectrum of transplantable mouse tumors*

Tumor	ATCase activity (nmols of CA†/mg of protein/hr)	Sensitivity to PALA in vivo	For data on antitumor activity see—
Mouse ovarian teratocarcinoma	108 ± 18	+	Table 1
Lewis lung carcinoma	123 ± 73	+ + +	Refs 13, 18
Ehrlich ascites carcinoma	159 ± 38	+ +	Table 2
Colon carcinoma 26	182 ± 26	+ +	Ref 22
Glioma 26	208 ± 29	+ +	Ref 12
B16 melanoma	251 ± 37	+ +	Refs 12, 13
M5076 ovarian carcinoma	260 ± 123	+ + +	Table 1
Colon carcinoma 38	393 ± 51	+	Table 1
L1210 leukemia	405 ± 185	–	Ref 12
P388 leukemia	484 ± 168	–	Ref 12
P388/ara-C resistant	490 ± 90	+ +	Ref 3
P815 mastocytoma	604 ± 121	–	Ref 12
L5178Y/AR leukemia	610 ± 91	–	Ref 12

*Tumors were transplanted into mice as described either in the Methods section or in the refs. Tumors were homogenized and assayed for ATCase activity according to the procedure presented in the Methods section. Sensitivity to PALA in vivo is expressed as: + + + (complete tumor inhibition with tumor-free long-term survivors); + + (> 90% tumor inhibition and/or > 75% increase in lifespan with few tumor-free long-term survivors); + (70%–90% tumor inhibition and/or 40%–75% increase in lifespan); and – (< 70% tumor inhibition and/or < 40% increase in lifespan).
†Carbamyl-L-aspartate formed.

TABLE 3.—Inhibition by PALA of ATCase activity in representative rodent tumors*

Tumor	Sensitivity to PALA in vivo†	Kinetic parameters of substrates			
		Carbamyl phosphate		L-Aspartic acid	
		K_m (mM)	K_i, PALA (μM)	K_m (mM)	K_i, PALA (μM)
Lewis lung carcinoma	S	0.95 ± 0.01	0.12 ± 0.05	2.35 ± 1.35	0.13 ± 0.06
Ehrlich ascites carcinoma	S	1.50 ± 0.16	0.07 ± 0.02	1.49 ± 0.23	0.20 ± 0.04
Mouse ovarian teratocarcinoma	S	1.16 ± 0.04	0.08 ± 0.01	1.29 ± 0.83	0.28 ± 0.05
B16 melanoma	S	0.63 ± 0.01	0.10 ± 0.01	1.52 ± 0.67	0.14 ± 0.03
L5178Y/AR leukemia	R	0.98 ± 0.32	0.08 ± 0.03	1.61 ± 0.27	0.26 ± 0.08
L1210 leukemia	R	1.72 ± 0.20	0.16 ± 0.02	1.54 ± 0.15	0.35 ± 0.02

*Kinetic studies on the inhibition of ATCase were carried out according to the methods detailed in the Methods section. When L-aspartic acid was the variable substrate, the concentration of carbamyl phosphate was held fixed at 2.0 mM and the concentration of L-aspartic acid was varied at 8 points from 0.078 to 5.0 mM. When carbamyl phosphate was the variable substrate, the concentration of L-aspartic acid was held constant at 1.25 mM and the concentration of carbamyl phosphate was varied at 8 points from 0.0325 to 8.0 mM. Shown are the means of 2–3 experiments, each entailing quadruplicate determinations of each variable. It should be pointed out that the K_ms of these partially purified enzymes for carbamyl phosphate are markedly higher than the values reported in the literature: 1.8×10^{-6} M (ref 10). This discrepancy may reflect differences in the enzymes, differences in the analytic techniques used, or a biphasic (high K_m as well as low K_m) affinity of the enzyme.

†S = sensitive; R = resistant.

ence between ATCase activity and degree of sensitivity to PALA in solid tumors; the very sensitive M5076 ovarian carcinoma has higher ATCase activity than some less sensitive tumors, and the ovarian teratocarcinoma, which did not prove to be very sensitive to PALA, has the lowest enzyme activity of any of the tumors studied. Some overlap between sensitive and refractory tumors was evident; for example, the moderately sensitive colon carcinoma 38 has virtually the same ATCase activity as the completely refractory L1210 leukemia. The unusual collateral sensitivity of the ara-C-resistant subline of P388 leukemia to PALA is not due to a decrease in ATCase activity in the resistant subline. This enhanced sensitivity to PALA was not observed in a subline of L1210 leukemia resistant to ara-C (Johnson RK, unpublished observation). Although this study supports previous findings (9–11) that sensitivity to PALA is a function of lower ATCase activity, it is apparent that other factors play a role in response of tumors to PALA.

In the first place, it was hypothesized that the ATCase from sensitive tumors might have a greater affinity for PALA than the enzyme from refractory tumors. Kinetic measurements were undertaken to examine this conjecture, and the results are shown in table 3. The values for the K_i of PALA versus either carbamyl phosphate or L-aspartate were not dissimilar for sensitive and refractory tumors. Moreover, the substrate affinity constants and maximal velocities of ATCase did not vary in a manner which would explain the differential drug sensitivity of these tumors.

It was next hypothesized that resistance to PALA might stem from a more rapid restitution of ATCase in refractory as contrasted to drug-susceptible tumors following exposure to PALA in vivo. Enhanced restoration of enzyme activity could result from more rapid synthesis of new enzyme molecules or from more extensive displacement of PALA from extant ATCase molecules as a consequence, for example, of competitive binding by an augmented pool of carbamyl phosphate. Although these two alternatives were not directly tested, measurements were made of the rate of reappearance of ATCase activity following administration of PALA at a well-tolerated dose of 400 mg/kg. For this study, the extremely sensitive Lewis lung carcinoma was compared with the completely refractory L5178Y/AR leukemia (fig 1). PALA proved to have a dramatic and prolonged effect on ATCase activity in both neoplasms. Inhibition of 90% was evident at 1 and 24 hours after administration of the drug. Enzyme activity recovered slowly thereafter, with full recovery not evident until about 11 days after drug administration. Slow recovery of ATCase activity correlated with prolonged retention of significant inhibitory concentrations of PALA in both tumors (fig 1) as measured by an enzymatic assay (15). Similar results with respect to duration of ATCase inhibition and intracellular PALA concentration were observed with another PALA-refractory tumor, P388 leukemia (data not shown).

The above findings indicate that, at a high dose, the time course of disappearance of intracellular PALA and recovery from inhibition of ATCase are similar in sensitive and refractory cell lines. The next possibility examined was that intracellular drug levels and ATCase inhibition could be diminished in refractory cells at low doses of PALA as a reflection of poor PALA transport.

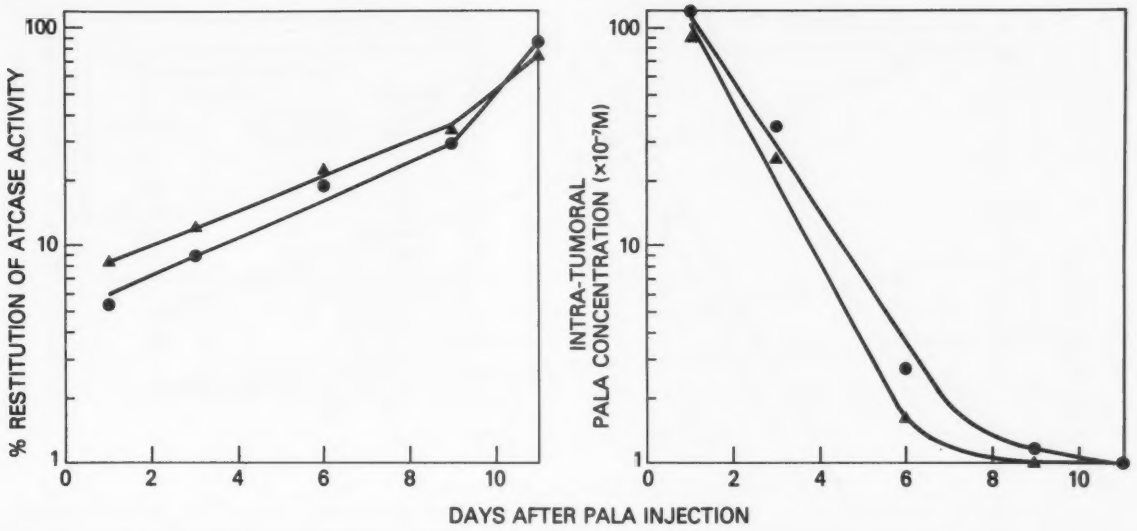
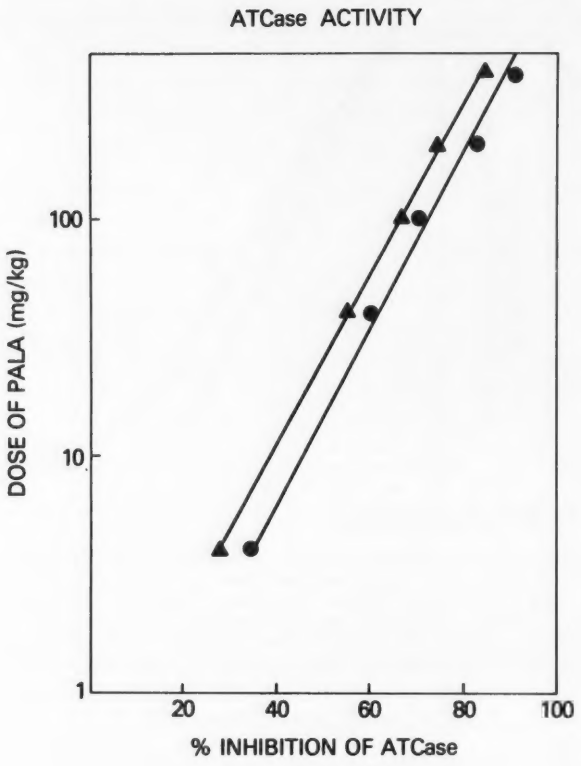


FIGURE 1.—Time course of restitution of ATCase activity and intratumoral concentrations of PALA in representative rodent neoplasms following a single dose of PALA. BDF_1 mice were implanted sc with either Lewis lung carcinoma (▲) or L5178Y/AR leukemia (●). 12 days later, tumor-bearing mice were injected with saline or 400 mg/kg of PALA. Animals were killed at the indicated time periods; tumors were collected and homogenized. Analysis of ATCase activity and PALA concentrations was carried out on the homogenates. The details of the entire procedure are presented in the Methods section. The values indicated in the figures are the means of quadruplicate measurements done on individual tumors from groups of 10 mice; variability between the measurements was < 5% in all cases.

Mice bearing established subcutaneous Lewis lung carcinoma or L5178Y/AR leukemia were treated with PALA at doses between 4 and 400 mg/kg. Twenty-four hours later, the tumors were excised and assayed for ATCase activity and for residual PALA by an enzymatic assay (15). There was a log-linear relationship between the dose of PALA and the degree of inhibition of ATCase at 24 hours (fig 2). Although the curves for the two tumors were quite similar, slightly more inhibition (about 5%) was evident in the drug-sensitive Lewis lung carcinoma at each dose level. The concentrations of PALA in both tumors were quite similar, ranging from about 1 μM following a dose of 4 mg/kg to ~100 μM after a dose of 400 mg/kg. Thus, limitation of PALA uptake does not appear to play an important role in resistance of tumors such as L5178Y leukemia to PALA.

In order to study PALA transport more directly, the uptake of labeled PALA in vitro was compared in sensitive and resistant ascites cell lines. Single-cell suspensions of ovarian teratocarcinoma, Ehrlich carcinoma, and L1210 leukemia were incubated with 250 μM PALA in tissue culture medium at 37°C. Uptake of PALA by the L1210 leukemia and the Ehrlich carcinoma was linear with regard to time and quite slow (fig 3). In contrast, the ovarian teratocarcinoma had a rapid initial rate of PALA uptake evident within the first few minutes of drug exposure. The uptake of PALA by the ovarian teratocarcinoma, after 4 hours of drug exposure, was eightfold higher than that found in the L1210 leukemia and fourfold higher than that found in the Ehrlich carcinoma. After 2 hours of exposure, the intracellular, bound and unbound, PALA concentration was only between 0.5% and 1.0% of the extracellular concentration. In ovarian teratocarcinoma cells, the intracellular PALA concentration was higher than in Ehrlich carcinoma cells, possibly because of the greater cell volume of the former (fig 3, *see* legend). Even though uptake of PALA was slow and minimal with respect to extracellular concentration, significant inhibition of ATCase was evident in all three cell lines by 15 minutes after exposure to 250 μM PALA; at 60 minutes, ATCase was inhibited by 72% in L1210 leukemia cells and 81% in ovarian teratocarcinoma and Ehrlich carcinoma cells.

The finding of similar intracellular levels of pharmacologically active drug in sensitive and resistant cells as shown in figures 1 and 2 would appear to mitigate against detoxification of PALA by tumor cells as a factor in resistance of tumors to the drug. To test this possibility directly, ovarian teratocarcinoma and L5178Y/AR leukemia cells were incubated with labeled PALA and any radioactivity taken up by the cells was evaluated chromatographically as described in the Methods section. Unchanged PALA accounted for 96% of the radioactivity associated with both cell lines. Furthermore, no metab-



INTRA-TUMORIAL PALA CONCENTRATIONS

Dose of PALA mg/kg	PALA Concentrations ($\times 10^{-7}$) in Tumors	
	Lewis Lung Carcinoma	L5178Y/AR
4	7.4	9.7
40	15.1	25.9
100	51.0	58.3
200	87.3	61.0
400	112.0	94.7

FIGURE 2.—Dose response of inhibition of ATCase activity by PALA and intratumoral concentrations of PALA in rodent neoplasms sensitive and resistant to PALA. Saline or indicated doses of PALA were injected ip into mice bearing subcutaneous Lewis lung carcinoma (▲) or L5178Y/AR leukemia (●). 24 hrs later, animals were killed, tumors were removed and homogenized, and analyses were carried out as detailed in the Methods section. The values indicated in the figures are the means of quadruplicate measurements done on individual tumors from groups of 10 mice; variability between the measurements was < 5% in all cases. PALA concentrations are molar.

olism of PALA by rat liver microsomes could be detected following incubation under aerobic or anaerobic conditions. These findings are in agreement with studies of the physiologic disposition of PALA in animals, in that metabolism of the drug has not been detected in mammalian tissues (20).

In the absence of firm evidence that differences in the cellular pharmacology of PALA account for the differential susceptibility of tumors, it was hypothesized that differences in the salvage pathway for synthesis of pyrimidine nucleotides could account for differential sensitivity to this inhibitor of the de novo pyrimidine nucleotide biosynthetic pathway. Uptake, anabolism, and catabolism of pyrimidine nucleosides were investigated. The initial rate of uptake of labeled uridine was compared in ovarian teratocarcinoma and L1210 leukemia cells (fig 4). The PALA-sensitive ovarian teratocarcinoma exhibited a twofold to threefold greater V_{max} for uridine uptake than the refractory L1210 leukemia. There was no significant difference in the K_m for uridine transport between the two cell lines.

A greater capacity to anabolize exogenous pyrimidines or pyrimidine nucleosides could contribute to resistance to PALA by virtue of decreased dependence on the de novo pathway for nucleic acid synthesis. To evaluate this possibility, the phosphorylation of pyrimidine nucleosides and deoxynucleosides by homogenates of a number of tumors was measured (table 4). Although only a limited number of tumor types were examined, this study did reveal that pyrimidine nucleoside phosphorylating activities were generally higher in cells resistant to PALA. For example, in the refractory L1210 leukemia, the kinases responsible for phosphorylating uridine, cytidine, thymidine, deoxyuridine, and deoxycytidine exhibited

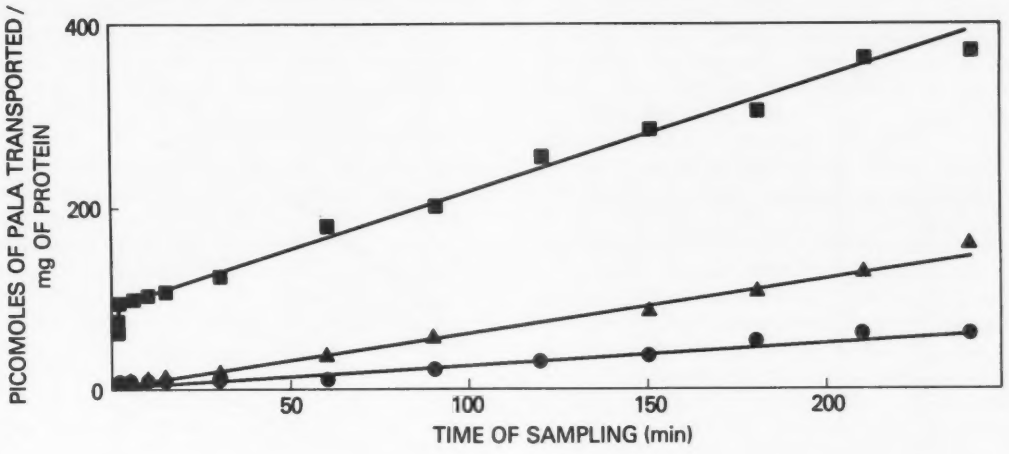


FIGURE 3.—Transport of PALA by sensitive and resistant tumors. Transport of PALA by L1210 leukemia (●), Ehrlich carcinoma (▲), and mouse ovarian teratocarcinoma (■) was carried out according to the procedure detailed in the Methods section. The value indicated in the figure is the mean of quadruplicate measurements done at each time point and is representative of 3 separate experiments; variability between the measurements was < 5% in all cases. The mean cell volumes of L1210 leukemia, mouse ovarian teratocarcinoma, and Ehrlich carcinoma cells were 12.5 ± 2.5, 45.0 ± 5.0, and 25.0 ± 5.0 μ^3 respectively. Electronic particle-sizing of the cells was carried out using a Coulter channelizer and counter.

specific activities that were ~4, 4, 2, 2, and 8 times greater respectively than comparable values for the Lewis lung carcinoma.

Using these same tumors, attempts were made to monitor the rate at which sensitive and resistant cells catabolized the pyrimidine ring; production of [^{14}C]-O_2 was used to measure the activity of the catabolic pathway (21). In all tumors, < 1% of added base or riboside ([2-^{14}C]-uracil, -uridine, or -deoxyuridine) was degraded to CO_2 during a 2-hour incubation at high cell density, and there was no difference apparent between PALA-responsive and -nonresponsive tumors in the rate of such catabolism.

DISCUSSION

These results support the contention that sensitivity of tumors to PALA is, at least in part, associated with low levels of ATCase activity. Kempe et al (11) showed that cultured cells with acquired resistance to the growth-inhibitory effects of PALA became resistant by virtue of overproduction of the carbamyl phosphate synthetase II-ATCase-dihydro-orotase enzyme complex. In these cell lines the initial twofold to fourfold elevation of ATCase activity over levels of activity present in the wild-type cell line was associated with a disproportionate decrease in sensitivity to PALA (about 40-fold). Further elevation of ATCase activity resulted in proportionately greater resistance to PALA. Johnson et al (7) found that, in cultured lines of PALA-sensitive solid tumors and PALA-refractory leukemias, relative resistance to the growth-inhibitory effects of PALA was associated with higher levels of ATCase activity and with a rapid rate of proliferation. In the present study ATCase activity was measured in homogenates of tumors taken directly from animals rather than in cultured cell lines. Solid tumors which are sensitive to PALA in vivo tended to have lower levels of ATCase activity than PALA-refractory leukemias. Generally it could be stated that tumors with high levels of ATCase activity (> 400 nmols of carbamyl-L-aspartate formed/mg of protein/hour in crude homogenates) will be refractory to PALA. An exception to this generalization is the ara-C-resistant subline of P388 leukemia, which is collaterally sensitive to PALA and yet has the same ATCase activity as the parental PALA-refractory leukemia. This tumor is also collaterally sensitive to other pyrimidine antimetabolites such as 5-fluorouracil and pyrazofurin (Schabel FM, Jr, Southern Research Institute, personal communication). The basis for the collateral sensitivity of the ara-C-resistant P388 leukemia to these agents is not known.

The finding of low ATCase activity in a given tumor does not predict sensitivity to PALA in vivo. Tumors with quite low levels of ATCase (for example, the ovarian teratocarcinoma) may prove less sensitive to PALA than tumors with considerably higher levels of enzyme activity

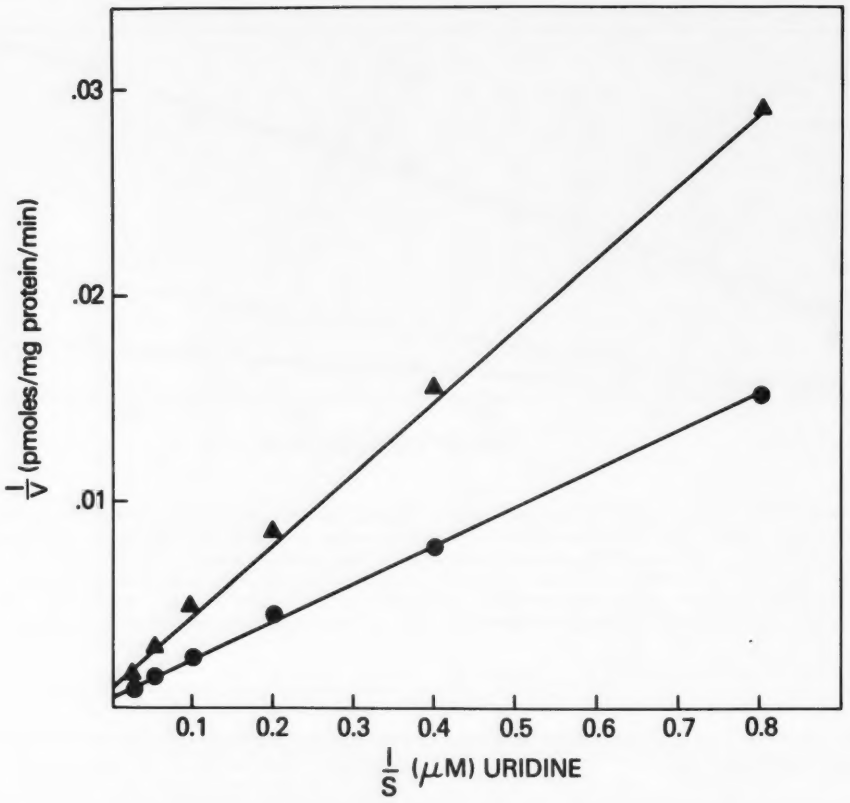


FIGURE 4.—Uptake of uridine by L1210 leukemia and mouse ovarian teratocarcinoma cells. L1210 leukemia (▲) or mouse ovarian teratocarcinoma (●) cells were prepared as indicated in the Methods section. Cells were incubated with [5,6-^{3}H]-uridine (1.25–40.0 μM) for 18 secs at 37°C and uptake then was terminated by centrifugation as detailed in the Methods section. The value indicated in the figure is the mean of quadruplicate measurements done at each concentration of uridine; variability between the measurements was < 5% in all cases. The lines of best fit were derived from linear regression analysis. K_m and V_{max} of 30 μM and 846 pmols/min/mg of protein respectively were calculated for L1210 leukemia cells; K_m and V_{max} of 40.4 μM and 2212 pmols/min/mg of protein respectively were calculated for mouse ovarian teratocarcinoma cells.

TABLE 4.—In vitro phosphorylation of pyrimidine nucleosides and deoxynucleosides by tumor homogenates*

Tumor	Sensitivity to PALA	In vitro phosphorylation (nmols of nucleotide formed/mg of protein/hr)				
		Uridine	Cytidine	Thymidine	Deoxyuridine	Deoxycytidine
Lewis lung carcinoma	+ + +	55.15 ± 7.99	13.34 ± 2.67	6.68 ± 1.24	4.08 ± 0.94	2.78 ± 0.32
Ehrlich ascites carcinoma	+ +	49.46 ± 4.34	11.16 ± 1.6	3.57 ± 0.29	3.12 ± 0.25	1.97 ± 0.20
Mouse ovarian teratocarcinoma	+	58.62 ± 8.31	30.92 ± 2.78	2.48 ± 0.86	1.87 ± 0.42	1.32 ± 0.23
L5178Y/AR leukemia	–	87.05 ± 19.16	25.24 ± 3.0	6.14 ± 1.44	4.17 ± 0.46	4.36 ± 1.10
L1210 leukemia	–	120.44 ± 43.78	65.24 ± 7.82	14.30 ± 2.94	7.24 ± 3.91	21.44 ± 7.99

*Homogenates from freshly excised tumors were prepared as detailed in the Methods section. The homogenates were spun at 12,000 × *g* for 6 mins, and the supernatants were incubated with the labeled precursors for 15 mins at 37°C and processed according to the procedures detailed in the Methods section.

(such as the M5076 ovarian carcinoma). Factors other than ATCase activity clearly play a role in the sensitivity of tumors to PALA. From the studies presented in this paper, a number of factors which could conceivably contribute to PALA sensitivity (or resistance) would appear to have little or no importance in differential response of tumors to this drug: (*a*) ATCase from sensitive tumors did not have a greater affinity for PALA than enzyme from refractory tumors; (*b*) ATCase activity was not reconstituted more rapidly in refractory than in sensitive tumors; (*c*) intracellular levels of PALA and the resultant degree of inhibition of ATCase achieved in sensitive and refractory tumors were similar; thus diminished PALA transport or differential intracellular sequestration (binding) of PALA to prevent inhibition of ATCase is not associated with PALA resistance; (*d*) PALA was not preferentially detoxified by refractory tumor cells; in fact, no metabolism of PALA could be detected; and (*e*) differences in catabolism of pyrimidines or pyrimidine nucleosides were not evident.

Although PALA uptake was found to be very slow in vitro as shown in figure 3, it is evident from the studies shown in figures 1 and 2 that intratumoral concentrations of PALA several orders of magnitude greater than its K_i can be achieved in vivo following readily tolerated doses of the drug. The concentration of PALA in sensitive and refractory tumors was essentially the same; thus, differential uptake of PALA by tumors would not appear to be related to degree of sensitivity to the drug. It should be stressed that the absolute levels of inhibition of ATCase shown in figures 1 and 2 are arbitrary values determined by the concentration of carbamyl phosphate used in the assay, since this precursor is competitive with PALA. The carbamyl phosphate concentration used, 4 mM, is quite high; thus, the reported levels of inhibition could be considerably lower than those which exist in situ. To determine the level of inhibition in situ, one would have to know the concentration of carbamyl phosphate at the active site of ATCase. Since carbamyl phosphate synthetase II and ATCase exist in a complex, channeling of carbamyl phosphate is a likely occurrence (11). Thus, intracellular levels of carbamyl phosphate may not reflect the concentration of this precursor at ATCase. Although the absolute levels of inhibition of ATCase in situ cannot be determined, the relative degrees of inhibition between the various tumors, time points, and PALA doses shown in figures 1 and 2 are valid since the same conditions were used in all assays. Levels of carbamyl phosphate at the active site of ATCase and thus ultimately the activity of carbamyl phosphate synthetase II could be important determinants of the sensitivity of tumors to PALA.

The activity of the salvage pathway could also influence the response of tumors to PALA. Measurements of the anabolism of pyrimidine nucleosides and deoxynucleosides by homogenates of sensitive and refractory tumors showed that the two leukemias which were evaluated had generally higher levels of pyrimidine nucleoside phosphorylating activity; this difference could contribute to insensitivity to PALA. It is important to note that the transplantable tumor most sensitive to the therapeutic effects of PALA, Lewis lung carcinoma, can be readily rescued from the effects of PALA by exogenous uridine administered to tumor-bearing mice either parenterally (6) or orally (Johnson RK, unpublished observation). This finding indicates that even PALA-sensitive tumors have a functional salvage pathway. Perhaps tumors with a highly active salvage pathway can effectively utilize the low levels of pyrimidine nucleotide precursors in the circulation. Combination therapy studies of PALA with the potent inhibitor of pyrimidine nucleoside uptake, 6-*p*-nitrobenzylthiopurine riboside (22), revealed synergistic host toxicity and somewhat greater therapeutic activity than PALA alone in mice bearing B16 melanoma (Johnson RK, unpublished observation).

The association of PALA sensitivity with low ATCase activity is probably due to the fact that activity of ATCase is in considerable excess compared to other enzyme activities in the de novo pyrimidine nucleotide biosynthetic pathway (23). Very extensive inhibition of ATCase would be necessary before this reaction becomes rate-limiting for the pathway. In cells with high levels of ATCase activity, adequate inhibition of the enzyme might not be achieved following maximally tolerated doses of PALA. An assay for inhibition of ATCase in whole cells would be useful for studying this point. The incorporation of labeled bicarbonate or L-aspartate into pyrimidine nucleotides (3) or effects of PALA on accumulation of orotate and orotidine in pyrazofurin-treated cells (24) could shed light on the differential effects of PALA in sensitive and refractory cells.

REFERENCES

1. COLLINS KD, and STARK GR. Aspartate transcarbamylase interaction with the transition state analog *N*-(phosphonacetyl)-L-aspartate. J Biol Chem 246:6599–6605, 1971.
2. HOOGENRAAD NJ. Reaction mechanism of aspartate transcarbamylase from mouse spleen. Arch Biochem Biophys 161:76–82, 1974.
3. SWYRYD EA, SEAVER SS, and STARK GR. *N*-(Phosphonacetyl)-L-aspartate, a potent transition state analog inhibitor of aspartate transcarbamylase, blocks proliferation of mammalian cells in culture. J Biol Chem 249:6945–6950, 1974.
4. TSUBOI KK, EDMUNDS HN, and KWONG LK. Selective inhibition of pyrimidine biosynthesis and effect on proliferative growth of colon cancer cells. Cancer Res 37:3080–3087, 1977.
5. ADAMSON RH, SIEBER SM, THORGEIRSSON SS, ET AL. Studies on the antitumor, embryotoxic and mutagenic effects of *N*-(phosphonacetyl)-L-aspartic acid (PALA). Proc Am Assoc Cancer Res and ASCO 19:649, 1978.
6. JOHNSON RK. Reversal of toxicity and antitumor activity of *N*-(phosphonacetyl)-L-aspartate by uridine or carbamyl-DL-aspartate in vivo. Biochem Pharmacol 26:81–84, 1977.

7. Johnson RK, Swyryd EA, and Stark GR. Effect of *N*-(phosphonacetyl)-L-aspartate on murine tumors and normal tissues in vivo and in vitro and the relationship of sensitivity to rate of proliferation and level of aspartate transcarbamylase. Cancer Res 38;371–378, 1978.
8. Johnson RK, Inouye T, Goldin A, et al. Antitumor activity of *N*-(phosphonacetyl)-L-aspartic acid, a transition-state inhibitor of aspartate transcarbamylase. Cancer Res 36:2720–2725, 1976.
9. Schabel FM, Jr, Laster WR, Jr, and Rose WC. Experimental chemotherapy and tumor cell kinetics: overview of experimental tumor systems. *In* Treatment of Lung Cancer (Muggia FM, and Rozencweig M, eds). New York, NY, Raven Press, 1978. In press.
10. Coleman PF, Suttle P, and Stark GR. Purification from hamster cells of the multifunction protein that initiates de novo synthesis of pyrimidine nucleotides. J Biol Chem 252:6379–6389, 1977.
11. Kempe TD, Swyryd EA, Bruist M, et al. Stable mutants of mammalian cells that overproduce the first three enzymes of pyrimidine nucleotide biosynthesis. Cell 9:541–550, 1976.
12. Sweeney MJ, Hoffman DH, and Poore GA. Enzymes in pyrimidine biosynthesis. *In* Advances in Enzyme Regulation (Weber G, ed). New York, NY, Pergamon Press, vol 9, 1970, pp 51–63.
13. Young JE, Prager MD, and Atkins IC. Comparative activities of aspartate transcarbamylase in various tissues of the rat. Proc Soc Exp Biol Med 125:860–862, 1967.
14. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254, 1976.
15. Cooney DA, Karlowicz MG, Cubillan J, et al. An enzymatic technique for measuring *N*-phosphonacetyl-L-aspartic acid in tissues. Cancer Treat Rep 62:1503–1507, 1978.
16. Litterst CL, Mimnaugh EG, Reagan RL, et al. Comparison of in vitro drug metabolism by lung, liver and kidney of several common laboratory species. Drug Metab Dispos 3:259–265, 1975.
17. Yamamoto CM, Lauzon GJ, and Paterson RP. Toxicity of combinations of arabinosylcytosine and 3-deazauridine toward neoplastic cells in culture. Biochem Pharmacol 27:181–186, 1976.
18. Cooney DA, Milman HA, and Truitt R. Disposable and versatile vessels for microdiffusion studies. Anal Biochem 41:583–589, 1971.
19. Brockman RW, Rose LM, Shaddix SC, et al. Biochemical aspects of chemotherapy of mouse neoplasms with *N*-(phosphonacetyl)-L-aspartate (PALA). Fed Proc 37:1693, 1978.
20. Chadwick M, Silveira DM, MacGregor JA, et al. Physiological disposition of PALA in several species. Proc Am Assoc Cancer Res and ASCO 19:726, 1978.
21. Canellakis ES. Pyrimidine metabolism. III. The interaction of the catabolic and anabolic pathways of uracil metabolism. J Biol Chem 227:701–709, 1957.
22. Paterson ARP, Naik SR, and Cass CE. Inhibition of uridine transport in HeLa cells by nitrobenzothioinosine and related compounds. Mol Pharmacol 13:1014–1023, 1977.
23. Sweeney MJ, Parton JW, and Hoffman DH. Biosynthesis of uridine-5′-monophosphate in rat liver and Morris hepatomas. *In* Advances in Enzyme Regulation (Weber G, ed). New York, NY, Pergamon Press, vol 12, 1973, pp 385–397.
24. Moyer JD, and Handschumacher RE. Selective *N*-phosphonacetyl-L-aspartate induced alterations of pyrimidine biosynthesis and nucleotide concentrations in mice. Fed Proc 37:243, 1978.

Role of Plasma Carcinoembryonic Antigen in Evaluating Patients With Breast Cancer Treated With Adjuvant Chemotherapy [1,2,3]

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SUMMARY

Serial plasma carcinoembryonic antigen (CEA) levels were determined in 84 patients with breast cancer who were receiving postsurgical adjuvant chemoimmunotherapy or adjuvant chemotherapy. CEA values were correlated with clinical status, scintiscans, alkaline phosphatase, LDH, and SGOT. CEA values > 2.5 ng/ml were considered abnormal. Thirty patients had normal serial CEA values; all remain disease-free. In 54 patients one or more abnormal CEA values were recorded; nine of 54 developed overt metastatic disease. Relapses occurred in four of 38 patients in whom values up to 5 ng/ml were recorded, in three of 14 patients in whom values up to 10 ng/ml were recorded, and in two of two patients in whom values > 10 ng/ml were recorded.

[Cancer Treat Rep 63:1303–1309, 1979]

A tumor-associated antigen, carcinoembryonic antigen (CEA), was first described by Gold et al (1). High concentrations were detected in the plasma of patients with colorectal neoplasms. Following the development of more sensitive radioimmune assay techniques for its assessment, elevated plasma CEA levels were detected in various neoplastic disorders. Elevation of CEA levels occurs in 80% of patients with metastatic breast cancer (2,3), and between 27% and 55% of patients with primary nonmetastatic breast cancer have abnormal values during the postoperative disease-free period (2–4). In breast cancer a high correlation between the level of circulating CEA and the disease activity was observed (5).

Changing concepts about the nature of breast cancer, improved results achieved in the treatment of women with metastatic breast cancer, and the remarkable impact of adjuvant therapy on the new concept of curative treatment for primary breast cancer require studies of markers which can help identify high-risk patients and can also help select the duration and intensity of treatment.

This study was undertaken to determine the role of CEA as a marker in patients with breast cancer treated with adjuvant chemotherapy or chemoimmunotherapy.

MATERIALS AND METHODS

Using the Hansen radioimmune assay, serial plasma CEA levels were determined at regular intervals in 84 patients with histologically confirmed breast cancer. CEA values > 2.5 ng/ml were considered abnormal. Intra-assay variation of 40 separate kits was determined statistically by means of paired t statistics ($0.25 > P > 0.10$). No significant intra-assay differences were obtained. Inter-assay evaluations were similarly evaluated with a similar result ($0.25 > P > 0.01$).

All patients had histologic involvement of ipsilateral axillary nodes at the time of primary mastectomy but no distal metastases. Distal metastases were excluded by the following criteria: no clinical evidence of residual tumor, normal chest and skeletal roentgenograms (tomograms where indicated), normal xerogram of remaining breast, and normal liver and bone scans. (If an asymp-

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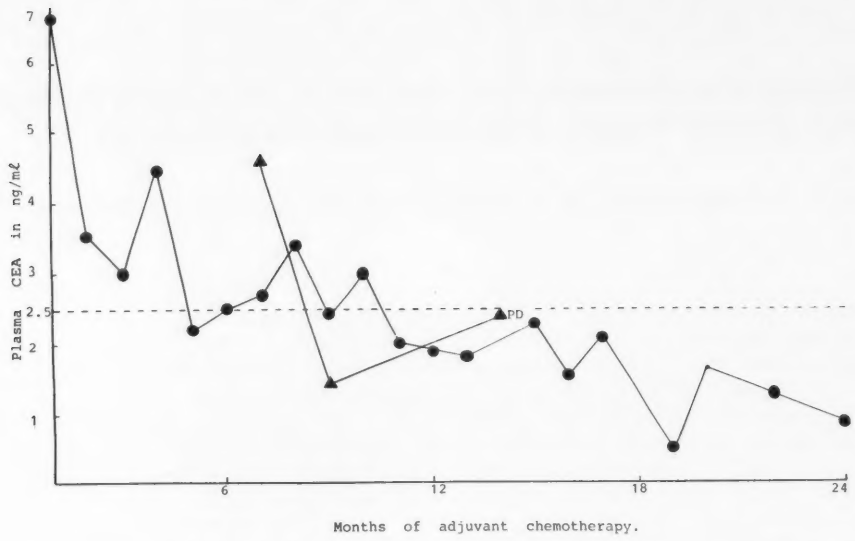

FIGURE 1.—Graph of 21 patients with initially elevated plasma CEA values which returned to < 2.5 ng/ml. ●——● = 20 patients who are still disease-free. ▲——▲ = 1 patient who developed bilateral lung metastases and died 1 mo after relapse.

tomatic patient with no other evidence of metastases had an equivocal bone scan, she was still considered to be eligible for the study.) Recurrence of disease was confirmed histologically where possible. The therapeutic regimens employed for adjuvant therapy included: (*a*) 28-day cycles of oral cyclophosphamide plus iv methotrexate plus iv 5-fluorouracil (CMF); (*b*) CMF plus methanol-extracted residue of BCG given intradermally; or (*c*) CMF plus vincristine plus oral prednisone. Adjuvant treatment was started within 1 month of mastectomy and continued for 2 years. Therapy during the second year consisted of CMF given at 2-month intervals.

The patients were seen frequently and were evaluated at monthly intervals by history, physical examination, cbc, serum protein electrophoretic pattern, serum alkaline phosphatase, LDH, SGOT, bilirubin, calcium, phosphorus, urea, uric acid, and creatinine; chest roentgenograms were done every 3 months and skeletal roentgenograms and liver and bone scintiscans were done every 6 months. When recurrence was suspected all investigations were repeated more frequently. Plasma CEA levels were determined at 3-month intervals, but when abnormal values were noted the test was done more frequently. In half of the patients in this series CEA determinations were done from the beginning of adjuvant therapy. However, when the CEA project was started, half of the patients were already receiving adjuvant therapy. Since CEA determinations were also done on these patients, and since valid information was obtained, patients already receiving adjuvant therapy have also been included in this analysis. In patients remaining disease-free, the minimum followup period for CEA determinations is 12 months.

CEA values were correlated with clinical status, serum alkaline phosphatase, LDH, SGOT, roentgenograms, and scintiscans in order to determine whether this biologic marker is useful for predicting relapse in patients with breast cancer.

RESULTS

Serial CEA values were recorded over a 1–2-year period in 84 patients. In 30 (36%) of the 84 patients all determinations were within normal limits (0–2.5 ng/ml). In 54 (64%) of the patients one or more abnormal CEA values were recorded. Table 1 shows the CEA values and clinical status of the patients.

Values of 0–2.5 ng/ml (30 patients)

In nine of 30 patients with normal CEA values, transient elevations of alkaline phosphatase, LDH, or SGOT were noted. All 30 patients remain free of any evidence of recurrence.

Values up to 5.0 ng/ml (38 patients)

When curves were drawn of sequential CEA levels in 38 patients in whom values up to 5 ng/ml were recorded, four different patterns occurred:

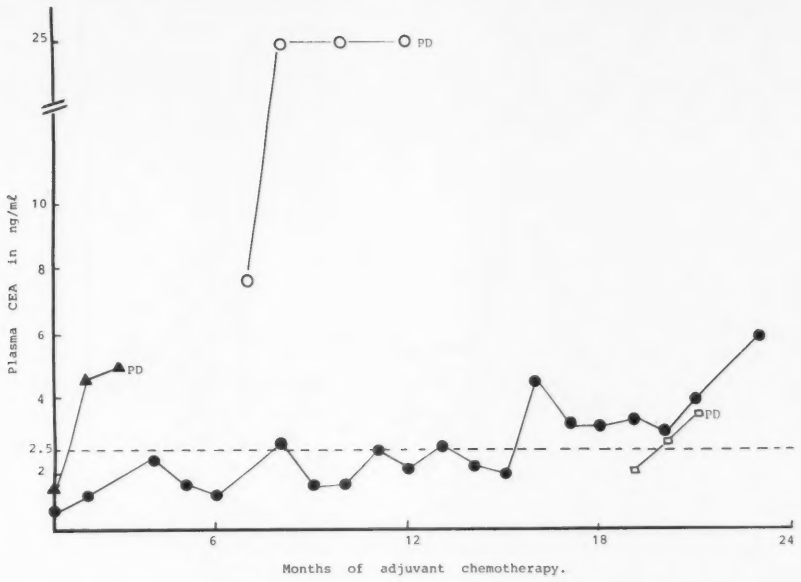

FIGURE 2.—Graph of 9 patients in whom CEA values progressively increased. ●——● = 6 patients who are still disease-free. ▲——▲ = 1 patient who developed skeletal metastases. She responded to subsequent chemotherapy and her CEA level decreased. ○——○ = 1 patient who developed hepatic metastases. The CEA remained > 25 ng/ml and she died 2 mos later. □——□ = 1 patient who developed hepatic, skeletal, and lymph node metastases. The CEA level gradually increased and was > 25 ng/ml at the time of death, 3 mos later.

a. Falling titer.—In 13 patients initial values (or values during the first 6 months of adjuvant chemotherapy) were between 2.6 and 5.0 ng/ml but decreased to normal levels within a mean of 7.8 months (range, 2–16 months). In these patients the values remained < 2.6 ng/ml during the rest of the adjuvant chemotherapy treatment period. One of these 13 patients developed recurrent disease at the 15th month with bilateral lung metastases and died 1 month later (fig 1).

b. Rising titer.—In seven patients normal initial values with a rise to > 2.5 ng/ml were seen. Elevation to above-normal levels occurred at a mean of 15 months (range, 2–20 months). Two of these seven patients developed recurrent disease: one with skeletal metastases at 3 months and the other with liver, lung, and skeletal metastases at 20 months. This latter patient died 2 months after relapse (fig 2).

c. Fluctuating titers or transient elevations.—Fluctuating titers or transient elevations > 2.5 ng/ml (but none > 5.0 ng/ml) occurred in 12 patients, five of whom were smokers. In one of the patients relapse occurred at the tenth month with histologically proven subcutaneous metastases (fig 3).

d. Persistent elevation.—Persistent elevation of up to 5.0 ng/ml was seen in six patients, three of whom were smokers. None of the six patients have developed recurrent disease (fig 4).

When CEA values for the 38 patients with values up to 5.0 ng/ml were compared with enzyme values recorded at the same time as the CEA, it was found that the four patients who developed recurrent disease had elevations of alkaline phosphatase and LDH before the CEA elevation occurred. In the patients who are still free of disease, elevations of alkaline phosphatase (usually transient) were noticed in seven patients, an elevation of LDH only was noticed in one patient, and elevations of both alkaline phosphatase and LDH were noticed in five patients. Serum electrophoretic pattern and serum transaminases remained normal in all 38 patients.

Values up to 10 ng/ml (14 patients)

As in the group of patients with values up to 5 ng/ml, four different patterns were observed when curves of sequential CEA values were drawn:

a. Falling titer.—In eight patients initial values (or values during the first 6 months) were between 5.1 and 10 ng/ml but decreased to normal levels within a mean of 9.8 months (range, 8–17 months) and remained < 2.6 ng/ml for the rest of the adjuvant chemotherapy period.

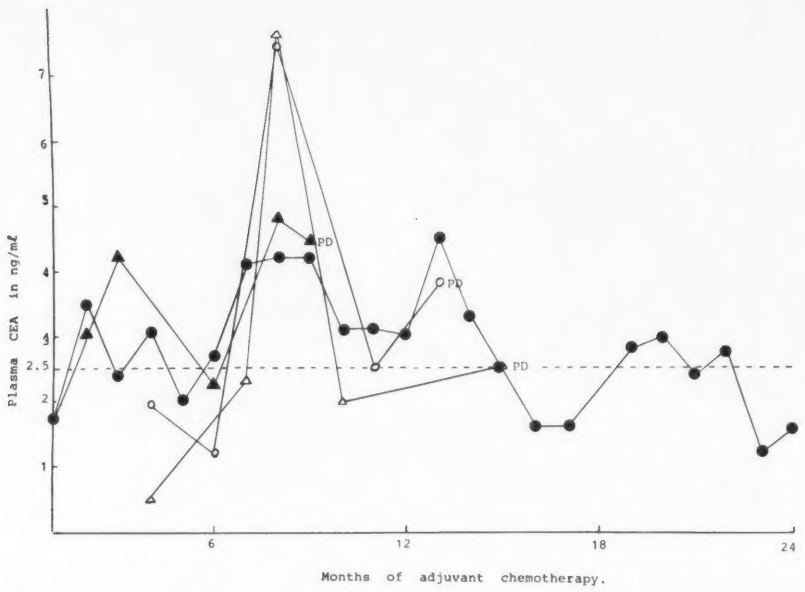

FIGURE 3.—Graph of 16 patients in whom CEA values fluctuated from < 2.5 ng/ml to above-normal values. ●——● = 13 patients who are still disease-free. ▲——▲ = 1 patient who developed subcutaneous metastases. CEA remained elevated and eventually decreased when response was achieved with subsequent chemotherapy. ○——○ = 1 patient who developed skin and subcutaneous metastases. CEA level remains elevated. △—— △ = 1 patient who developed lymph node metastases. CEA level remains < 2.5 ng/ml.

Two patients also had elevations of alkaline phosphatase and LDH and one patient had an elevation of alkaline phosphatase only. None of these patients developed recurrent disease (fig 1).

b. Rising titer.—One patient had initial normal CEA values with a gradual increase to > 2.5 ng/ml at 17 months and > 5 ng/ml at 23 months. Her LDH level remained normal, but her alkaline phosphatase and SGOT values became elevated concomitantly with the CEA elevations. She developed a suspicious bone scintiscan, but recurrence of disease has not yet been seen (fig 2).

c. Fluctuating titer.—Four patients had fluctuating titers. In two of the four patients relapse occurred at 16 months. One patient developed metastatic supraclavicular glands and the other patient developed skin and subcutaneous lesions. One of these two patients had an elevated LDH in addition to the abnormal CEA; the other had normal alkaline phosphatase, LDH, and SGOT levels. Two of the four patients with titers fluctuating between 0 and 10 ng/ml remain disease-free at 15 and 24 months respectively. Transient elevations of alkaline phosphatase and LDH were also recorded in one, while the other (a heavy smoker) had no abnormalities other than the elevations of CEA (fig 3).

d. Persistent elevations.—Persistent elevations between 2.5 and 6.8 ng/ml occurred in one patient who developed hepatic metastases at 21 months. All biochemical tests were normal until relapse occurred (fig 4). The patient died 3 months after relapse.

Values of > 10 ng/ml (two patients)

In this series only two patients had CEA values > 10 ng/ml; both developed recurrent disease. One patient had persistent elevations of alkaline phosphatase and CEA but had normal LDH levels. Her highest CEA value was 17 ng/ml, recorded 3 months before liver metastases were documented by isotope scan during the 19th month of adjuvant treatment (fig 4). The other patient remained completely free of any evidence of disease (no clinical or roentgenographic evidence and normal isotope scans of bone and liver). Her plasma CEA level, initially recorded during the seventh month of adjuvant treatment, was 7.8 ng/ml; it increased to > 25 ng/ml at the eighth month and remained > 25 ng/ml for 5 months before any other evidence of disease was apparent (fig 2). Six months after the first recording of an elevated CEA, suspected relapse was confirmed when the patient developed gross hepa-

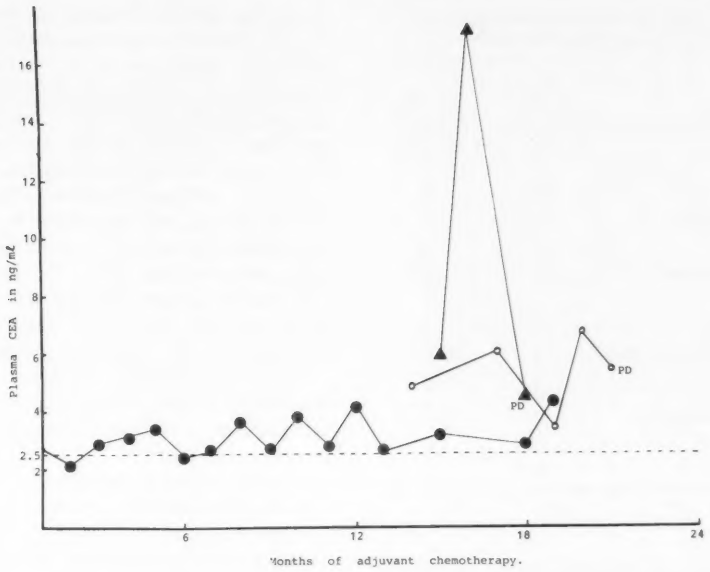

FIGURE 4.—Graph of 8 patients in whom persistently above-normal CEA values occurred. ●——● = 6 patients who are still disease-free (3 are smokers). ▲——▲ = 1 patient who developed hepatic metastases. She responded to subsequent chemotherapy and is still in remission 1 yr after relapse. CEA level remains just > 5 ng/ml (she is a heavy smoker). ○——○ = 1 patient who developed hepatic metastases. She did not respond to subsequent therapy. However, her CEA level dropped to 1.4 ng/ml immediately before death 3 mos after relapse.

tomegaly; isotope scans and abdominal sonography confirmed liver metastases. Elevations of alkaline phosphatase and LDH occurred for the first time at this stage. Her chemotherapeutic regimen was changed, but the disease progressed rapidly, with death due to liver metastases occurring 2 months after relapse.

Statistical analysis of initial values recorded in all 84 patients showed that there was only a 1% chance of CEA values > 7 ng/ml occurring in these patients, and that a patient with a value > 7 ng/ml could be considered a high-risk individual (this probability was calculated with the log-normal distribution). A statistically significant number of patients had a decreasing CEA titer (chi-square test, $P < 0.05$). The majority of patients who developed recurrent disease did so when their CEA levels were rising or persistently elevated, but this was not statistically significant ($P > 0.05$).

Of the 84 patients in the present series, two of two who showed an elevation of CEA > 10 ng/ml developed recurrent disease (100%), three of 14 who showed an elevation of 5–10 ng/ml developed recurrent disease (20%), and four of 38 who showed only mild elevation of 2.5–5.0 ng/ml developed recurrent disease (10%). Of the 30 patients who did not show an elevation of CEA > 2.5 ng/ml, none developed recurrent disease (table 1).

TABLE 1.—CEA determinations in patients receiving adjuvant chemotherapy for breast cancer

Clinical status	No. of patients with CEA values (ng/ml) of—				
	0–2.5	0–5	0–10	0–25	> 25
NED*	30	34	11	0	0
PD†	0	4	3	1	1

*NED = no evidence of disease.
†PD = progressive disease.

Of the nine patients who developed recurrent disease (and who all showed elevation of CEA), one had concomitant elevation of alkaline phosphatase, one had concomitant elevation of LDH, and four had concomitant elevation of both LDH and alkaline phosphatase (table 2). Therefore, six of the nine patients had other concomitant biochemical abnormalities and three had neither alkaline phosphatase nor LDH abnormalities. When CEA and alkaline phosphatase and/or LDH are abnormal the chances that this may predict relapse are greater than if CEA alone is elevated. However, this was not statistically significant ($P > 0.85$).

At some time during the observation period a further 45 patients had elevations of CEA (2.6–5 ng/ml in 34

TABLE 2.—Results of 3 tests recorded in 84 patients receiving adjuvant chemotherapy for breast cancer

Results of tests	No. of patients tested	No. who relapsed
All values normal	21	0
CEA only abnormal	28	3
CEA + alkaline phosphatase + LDH abnormal	12	4
CEA + alkaline phosphatase abnormal	12	1
CEA + LDH abnormal	2	1
Alkaline phosphatase only abnormal	7	0
LDH only abnormal	0	0
LDH + alkaline phosphatase abnormal	2	0
Total	84	9

patients; 5.1–10 ng/ml in 11 patients) but did not develop overt metastatic disease.

DISCUSSION

In breast cancer a major need exists for a biochemical marker. Adjuvant chemotherapy is most likely to be successful if patients with progressive disease can be identified, and if intensity and duration of treatment can be determined. Plasma CEA as a monitor of clinical progression in patients with gastrointestinal malignancy has been extensively investigated, and a 90% correlation of progression of disease with rising CEA has been documented. Indeed, in 30% of patients with gastrointestinal malignancy a CEA rise may indicate progression of disease ≥ 6 months before it could be detected by standard clinical methods (6). Tormey[7] has suggested that a similar early rise of plasma CEA, well before clinical evidence of relapse, may occur in some patients with breast cancer. In our experience, in advanced breast cancer, a high correlation exists between disease activity and plasma CEA levels (5). It therefore seemed feasible that this marker could be utilized when following patients treated with adjuvant chemotherapy for breast cancer.

In studying the effect of adjuvant chemotherapy for breast cancer on the plasma CEA levels, we found that a statistically significant number of patients had decreasing CEA values ($P < 0.05$). It is probable that this decrease from elevated to normal values may reflect eradication of occult metastases. However, in two of nine patients who developed recurrent disease, the relapse occurred when CEA values had returned to normal levels. At the present time no statistically significant relationship between the titers of CEA and relapse can be delineated. Bagshawe et al (7) observed transient elevations shortly after institution of antitumor therapy, presumably reflecting cell death with CEA release. Factors other than tumor burden are known to affect plasma CEA levels. Several investigators have emphasized the importance of hepatic function in the metabolism of CEA in both animals and man (8,9). We found no consistent relationship between CEA recordings and liver function tests. In more than half of the patients with abnormal CEA values all liver function tests were within normal limits, and one third of the patients with normal CEA had transient abnormal liver function tests.

Persistent elevations of CEA up to 5 ng/ml have been reported in patients who are heavy smokers. In some of our patients persistent elevations of 2.6–5 ng/ml, and transient elevations of > 2.5 and even > 5 ng/ml, might have been caused by smoking habits.

In the nine patients who developed recurrent disease two had values of > 10 ng/ml, three had values of 5.1–10 ng/ml, and four had only moderate elevations of 2.6–5 ng/ml. The finding that only six of the nine patients had other concomitant biochemical abnormalities shows better correlation between abnormal CEA values and prediction of relapse than for either alkaline phosphatase or LDH.

In light of these findings, we conclude that CEA determinations are of value, but cannot be considered a sufficiently specific marker for patients receiving adjuvant chemotherapy for breast cancer. However, the importance of CEA determinations in patients with metastatic breast cancer (5) argues for the continuation of CEA monitoring in the adjuvant situation. Although CEA by itself (like any other laboratory investigation) may have limited value, when a persistently elevated or a rising CEA titer is seen together with abnormal levels of other tests such as alkaline phosphatase and LDH, investigators should be alerted to carry out a more intensive search for metastases.

[7]Tormey DC. Personal communication, 1977.

REFERENCES

1. GOLD P, and FREEDMAN SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 122:467–481, 1965.
2. STEWARD AM, NIXON D, ZAMCHECK N, ET AL. Carcinoembryonic antigen in breast cancer patients: serum levels and disease progress. Cancer 33:1246–1252, 1974.
3. TORMEY DC, WAALKES TP, SNYDER JJ, ET AL. Biological markers in breast carcinoma. III. Clinical correlations with carcinoembryonic antigen. Cancer 39:2397–2404, 1977.
4. BORTHWICK NM, WILSON DW, and BELL PA. Carcinoembryonic antigen (CEA) in patients with breast cancer. Eur J Cancer 13:171–176, 1977.
5. FALKSON HC, VAN DER WATT JJ, PORTUGAL MA, ET AL. Carcinoembryonic antigen in patients with breast cancer. Cancer 42:1308–1313, 1978.

6. HOLYOKE ED, CHU TM, and MURPHY GP. CEA as a monitor of gastrointestinal malignancy. Cancer 35:830–836, 1975.

7. BAGSHAWE KD, ROGERS GT, SEARLE F, ET AL. Blood carcinoembryonic antigen, Regan isoenzyme, and human chorionic gonadotrophin in primary mediastinal carcinoma. Lancet 1:210–211, 1973.

8. SHUSTER J, SILVERMAN M, and GOLD P. Metabolism of human carcinoembryonic antigen in xenogeneic animals. Cancer Res 33:65–68, 1973.

9. PRIMUS FJ, WANG RH, and GOLDENBERG DM. Localization of human GW–39 tumors in hamsters by radiolabeled heterospecific antibody to carcinoembryonic antigen. Cancer Res 33:2977–2982, 1973.

Evaluation of Whole-Body Hyperthermia as an Adjunct to Chemotherapy in Murine Tumors [1,2]

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SUMMARY

Whole-body hyperthermia (WBH), induced in an atmosphere of high relative humidity, was evaluated alone and in combination with chemotherapy for its effect upon mice bearing one of several different tumors. Two different WBH protocols were used. The first involved administering drug to mice immediately prior to inducing WBH (Rx → WBH). Using this protocol, preliminary drug toxicity experiments in non-tumor-bearing mice receiving WBH ("heated" mice) indicated that the single-dose LD10s of cyclophosphamide (CPA) and methyl-CCNU (MeCCNU), and the multiple-dose LD10 of vincristine (VCR) were about 60%–80% of the LD10s in unheated mice. Differences in drug sensitivities between heated (Rx → WBH) and unheated mice were either minimal or not detected in mice treated with single doses of actinomycin D (act-D), Adriamycin (ADR), or CCNU, or multiple doses of 5-fluorouracil (5-FU) or palmO-ara-C (P-ara-C). Mice bearing either P388 leukemia, Lewis lung carcinoma, Ridgway osteogenic sarcoma, mammary adenocarcinoma 16C, or colon carcinoma 38 were subjected to 45 minutes of heating (60 minutes on occasion), either once or q4days × 3, during which time their body temperature reached and was maintained at ≥ 41.5°C (maximum mean temperature, 42.4°C) for approximately 20 minutes (minimum, 15 minutes). This approximate maximum degree and duration of WBH alone failed to alter the mortality pattern among mice bearing these tumors. Treatment with ADR, act-D, CCNU, MeCCNU, CPA, 5-FU, P-ara-C, or VCR immediately before inducing WBH was evaluated in one or more tumor systems. With the possible exception of act-D followed by WBH used to treat mice bearing P388 leukemia, WBH failed to potentiate the antitumor activity of any of these drugs. The second heating protocol involved the induction of WBH in tumor-bearing mice (to a rectal temperature of 41.0°C) prior to drug treatment and maintenance of the hyperthermic state at ≥ 41.0°C (maximum mean temperature, 42.5°C) for 30 minutes following drug administration (Rx during WBH). Experiments conducted in this manner, involving act-D or ADR against P388 leukemia, and CCNU against Lewis lung carcinoma, failed to demonstrate improved therapeutic responses of these tumors to these drugs by the addition of WBH. Studies on the possible effect of WBH on the therapeutic activity of selected representatives of the major classes (both chemical and mechanism of action) of clinically useful anticancer agents (ADR, act-D, CCNU, MeCCNU, CPA, 5-FU, P-ara-C, and VCR) against selected, drug-sensitive, transplantable murine tumors (P388, Ridgway osteogenic sarcoma, Lewis lung carcinoma, mammary adenocarcinoma 16C, and colon carcinoma 38) failed to demonstrate any reproducible and/or substantial enhancement of chemotherapeutic activity by WBH using the experimental procedures described.

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The use of both local and whole-body hyperthermia (WBH) as a primary treatment for cancer, and as an adjunct to other primary modalities (eg, surgery, radiation, and chemotherapy), is currently being evaluated with much renewed interest. The basis for thermally induced cell damage, including the reported selective toxicity of hyperthermia for malignant cells, is insufficiently understood (1–7). Selective heat sensitivity of cancer cells is the subject of a recent monograph containing reports by many of the active workers in the field (1). Included in it are chapters involving summaries of experimental and clinical applications of hyperthermia, both alone or as a part of combined modality regimens. Within the past several years, local hyperthermia induced by immersion in a water bath, radiant heat, shortwave diathermy, radiowaves, or microwaves has been reported to be variably effective in treating rats bearing the Yoshida sarcoma (1,8,9), rabbits bearing VX-2 carcinoma (1,10), and mice bearing mammary tumors (11–17). Combined modality therapies with local hyperthermia plus X-irradiation (1,13), or drugs (1,9,14–16,18,19), have reportedly been useful in treating these as well as other tumors (1,20,21).

Experimental studies with WBH have been reported less frequently than those involving local hyperthermia. Treatment of rabbits bearing the VX-2 carcinoma with WBH (using radiant heat) was not as effective as local hyperthermia, possibly due to inadequate deep-body hyperthermia that may have been stimulatory to metastatic disease and capable of depressing any immune response (1,10). WBH following total-body X-irradiation (300–800 rads) of mice bearing L1210 leukemia reportedly enhanced radiation damage of tumor cells based upon a bioassay for viable residual leukemic cells; however, the greatest tumor cell kill was obtained at radiation doses (eg, 600 rads) that exceeded the LD50 in non-tumor-bearing mice (22). Similarly, a regimen of WBH plus Adriamycin (ADR), used to treat mice bearing a transplanted mammary carcinoma, was lethal for all the mice (14). A compilation of these recent experimental results is shown in table 1.

Clinical trials using local hyperthermia have involved perfusion of the extremities for the treatment of melanomas (2,23), sarcomas, and squamous cell carcinomas (2). Local hyperthermia was also used in the treatment of bladder carcinoma by lavage of that organ with hot isotonic fluid (24). Pettigrew et al (25) treated 51 patients "in terminal stages of cancer" with WBH induced by application of molten wax, either alone or in combination with cyclophosphamide (CPA), vincristine (VCR), 5-fluorouracil (5-FU), or melphalan. Useful objective and subjective responses were reported, with the most sensitive tumors being those of the gastrointestinal tract and sarcomas. Many other reports of clinical trials involving hyperthermia have been reviewed recently (1).

Most data available from published reports on WBH plus chemotherapy against human or animal tumors have not objectively established therapeutic synergism by these combined treatment modalities (1). Since reliable methods for quantitating tumor cell kill by drugs are available (26,27), we designed and carried out therapeutic trials using WBH alone and in combination with representatives of the major classes of clinically useful anticancer drugs against selected mouse tumors with well-defined and reproducible sensitivity to these drugs.

MATERIALS AND METHODS

The tumors, host mice, and drugs used in these studies are listed in table 2. With few exceptions, female mice, 6–12 weeks old, were used. Mammary adenocarcinoma 16C (MAM 16C) and colon carcinoma 38 (CC 38) were obtained from Dr. T. H. Corbett of this Institute and their characteristics and history have been previously described (28,29).

Hyperthermia

Two different heating protocols were used for WBH experiments. The basic methods used in inducing WBH in mice were recommended and developed by Dr. E. R. Atkinson (National Cancer Institute, Bethesda, Md) and initial pilot studies were performed under his indirect supervision.

First Heating Protocol (Rx → WBH)

WBH was induced in water-jacketed, 10.5-cubic foot incubators (Model E2-18M, Wedco, Inc, Silver Spring, Md) at temperatures of 38.7°C ± 0.1°C for all drug toxicity determinations and 38.8°C–38.9°C for subsequent antitumor chemotherapy evaluations. Relative humidity at or near 100% was maintained by forced circulation of the ambient air over 8 × 17-inch water reservoirs in each incubator. The incubators were housed in a small room in which the ambient temperature was 33°C–34°C.

While in the incubators, mice were housed five to a cage in 10 × 6 × 5-inch (length × width × height) plastic cages with hardwood chip bedding. Each cage had several holes drilled around the periphery approximately midway between the bottom and the top and a thin wire-mesh cage top to provide maximum ventilation. A maximum of 35 mice (36 mice including the "temperature-monitor" mouse, see below) were entered into the incubators for each exposure.

One additional mouse in each incubator was used to monitor deep-body temperature. This temperature-monitor mouse was lightly anesthetized with pentobarbital and a thermocouple probe (Model IT-1, Bailey Instruments, Inc, Saddle Brook, NJ) was inserted into the rec-

tum to a depth of about 12 mm and retained in place throughout the heating period. Rectal temperatures were measured by a digital thermometer (Model BAT-8C, Bailey Instruments, Inc) and recorded at 5-minute intervals. Air temperature inside the incubators was monitored using a "fast-response" (0.025-second time constant) microprobe (Model MT-3, Bailey Instruments, Inc). The thermometers were periodically calibrated using a saturated Na_2SO_4 solution with a known hydration temperature of 32.4°C. Neither food nor water was supplied to the mice while they were in the incubators.

Mice were given drug or diluent and immediately (≤ 3 minutes) placed in the incubator. The duration of exposure to elevated temperature in all studies reported was 45 minutes unless otherwise specified. Multiple-dose chemotherapy schedules included WBH following each drug injection. In most experiments, mice in each treatment group were evenly divided between two incubators. An experimental run was considered valid if: (*a*) a rectal temperature of 41.5°C was attained (in the temperature-monitor mouse) within 30 minutes of entry into the incubator; (*b*) no more than one of five heated non-tumor-bearing mice, given only drug diluent, died during the experimental observation period; and (*c*) the incubator air temperature varied by no more than 0.1°C from the designated desired incubator temperature during the 45-minute exposure. Any exceptions to these criteria are specifically stated in the results. Experimental observation times were at least 30 days after the end of treatment for drug toxicity experiments, 58 days after treatment for experiments involving leukemias, and 80–90 days after treatment for experiments involving solid tumors.

Second Heating Protocol (Rx During WBH)

The second protocol differed from the first in that mice were first heated in the incubators until the rectal temperature of the temperature-monitor mouse reached 41.0°C (in about 20 minutes), at which time, using rubber sleeves with gloves attached to plexiglass doors mounted on the incubators, injections of the drugs or diluents were given with the mice still in the incubators. Heating was continued for 30 minutes after injection. In this way, maximum blood levels of drug would probably be attained while the mice were experiencing maximally tolerated hyperthermia.

RESULTS

Response of Normal Mice to WBH

In preliminary studies, we observed that the rectal temperature responses of tumor-bearing mice during WBH were not different from those of non-tumor-bearing mice. We therefore used non-tumor-bearing mice in all subsequent experimental runs when monitoring rectal temperature responses. A graphic representation of the average rectal temperature responses to WBH of 48 mice used to monitor deep-body temperature during the drug Rx → WBH studies (incubator temperature, 38.8°C–38.9°C) is shown in figure 1. The temperature response curve obtained was very similar to that found among mice exposed to the slightly lower incubator temperature (38.7°C) used in preliminary drug toxicity experiments (discussed below). A rectal temperature of 41.5°C was reached in mice between 20 and 30 minutes (mean, 26 minutes) after placing them in the incubator in all experimental runs included for analysis. Thus, using Rx → WBH, deep-body temperature was maintained at ≥ 41.5°C for approximately the last 20 minutes (minimum 15 minutes) in each 45-minute interval after drug treatment. Maximum rectal temperatures, usually attained about 45 minutes after placing the mice in the incubator, were slightly > 42°C (mean, 42.4°C). Under these conditions, $AKD2F_1$ mice were more susceptible to the lethal effect of WBH than were the other hybrid mouse strains used. In keeping with the validation criterion (*see* Materials and Methods section) that no more than one of five diluent-heat control mice could die following WBH, only experiments, including those involving $AKD2F_1$ mice, that met this criterion were considered for analysis.

The average rectal temperature response of 12 mice used to monitor the six experiments performed using the second heating protocol (Rx during WBH) is shown in figure 2. The average maximum rectal temperature obtained was approximately 42.5°C, and an average rectal temperature of ≥41.0°C was maintained for the entire incubation period following drug injection. The same validating criteria that were used with the Rx → WBH studies were used here.

Comparative Drug Toxicity

The lethal toxicity of various anticancer agents in both heated and unheated mice is presented in table 3. Heated mice were those exposed to incubation temperatures of 38.7°C ± 0.1°C in drug toxicity experiments and those (drug toxicity control mice) exposed to 38.8°C–38.9°C in antitumor experiments, using the protocol in which hyperthermia was induced following drug administration (Rx → WBH). The drug toxicity data were compiled from one to four (usually three) experiments for each drug in each host strain. In each experiment, three drug doses in five to ten mice/dose were used and the LD10s for each drug tested were estimated from pooled group sizes varying from five to 50 mice/dose, usually ten to 30 mice/dose.

With respect to differences in LD10 for drugs given to unheated and heated mice, the most consistent pattern of altered sensitivity was found when single doses of CPA or methyl-CCNU (MeCCNU) were given to BDF_1 mice.

TABLE 1.—Survey of recent literature involving in vivo experimental results with hyperthermia

Tumor	Host	Local (L) hyperthermia or WBH	Heating method	Other therapy*	External temperature (°C)	Internal temperature (°C)		Exposure time	Treatment lethality	Reported therapeutic results	Ref No.
						Body	Tumor				
Yoshida sarcoma	Rat	L	Water bath	None	40.5	—	40.0	1 hr	—	Increased metastases. Smaller tumors (< 3.0 ml) responded; larger tumors caused earlier deaths.	8
Yoshida sarcoma	Wistar rat	L	Water bath	MDMS	42.5–42.7	40.0	42.0	1 hr	—	Heat alone effective. Heat and drug not as good as drug alone.	9
VX-2 carcinoma	Rabbit	L	Water bath	None	46.0	Normal	Mean, 42.7	75 mins, qd × 3	—	50% cured.	10
	Rabbit	WBH	Radiant heat	None	46–48	40.5–43.2	Mean, 42.6	75 mins, qd × 3	None	Some tumor regression and 1 of 14 cured.	
Mammary adenocarcinoma	C3H mice	L	Microwave	None	—	31–33	43.0–43.5	45 mins, q2days × 4	None	100% cured.	11
Mammary adenocarcinoma	C3H mice	L	Water bath	None	44.5	—	44.1	15, 39, 51, or 60 mins	6.5% overall	Tumor growth retardation; no cures.	12
Mammary adenocarcinoma	C3H mice	L	Water bath	X-ray	44.5	—	44.1	15 or 30 mins	—	Heat reduced the ED50 of X-ray.	13
Mammary carcinoma	$C3D2F_1$ mice	L	Shortwave diathermy	ADR	—	—	40.5 42.5	2 hrs 1 hr	38%–50%	Some cures with combinations but unacceptable toxicity.	14
	$C3D2F_1$ mice	WBH	Radiant heat	ADR	—	—	40.5	2 hrs	100%	Lethal.	
EMT-6 mammary sarcoma	BALB/c mice	L	Water bath	ADR and bleomycin	43.5	36.8 ± 0.2	42.8–43.1	1.8 hrs	—	Heat + ADR (or bleomycin) synergistic.	15
EMT-6 sarcoma	BALB/c mice	L	Water bath	Ro-07-0582	42–45	≤ 39.0	40–44	1 hr	12%	By bioassay, drug + intratumor temperature of ≥ 42.5°C was synergistic.	16
EMT-6 sarcoma; KHJJ carcinoma	BALB/c mice	L	Radiofrequency	None	—	36.0	43–44	5–40 mins	2%–3%	≥ 50% cure rates of both tumors.	17

EMT-6 sarcoma	BALB/c mice	L	Water bath	Am-B	43.5	—	43.0	40 mins	—	By bioassay, combination resulted in enhanced tumor cell kill (< 1-log difference).	18
EMT-6 sarcoma	BALB/c mice	L	Water bath	BCNU	43.0	≤ 38	Mean, 41.6; maximum, 42.4	1 hr	Minimal	Synergistic with regard to both growth delay and cures.	19
Ridgway osteosarcoma	$AKD2F_1$ mice	L	Heat lamp	X-ray	—	—	42.5	15 mins, q2days × 3	—	Heat alone: 0–50% ILS. Heat + X-ray synergistic, resulting in some cures.	20
SV40 fibrosarcoma	$C8F_1$ mice	L	Hot air	X-ray	45, 50, or 55	≤ 38.5	39–44	15, 30, or 45 mins, once or q7days × 2	—	Optimal regimens synergistic. Increased lifespan and some cures obtained.	21
L1210 leukemia	$C3D2F_1$ mice	WBH	Water bath	X-ray	41.5–42.0	41.5–42.0		20–60 mins	—	By bioassay, X-ray → WBH was found to enhance the killing of L1210 leukemia.	22

*MDMS = methylenedimethanesulfonate; ADR = Adriamycin; Ro-07-0582 = 1-(2-nitroimidazol-1-yl)-3-methoxy-2-propanol); Am-B = amphotericin B.

TABLE 2.—Summary of tumors, host mice, and drugs used in the present studies

Tumor	Host	Drugs*
P388 leukemia	BDF_1	Act-D, ADR, VCR
Ridgway osteogenic sarcoma	$AKD2F_1$	Act-D, P-ara-C
Lewis lung carcinoma	BDF_1	CPA, CCNU, MeCCNU
Mammary adenocarcinoma 16C	$B6C3F_1$	ADR
Colon carcinoma 38	$B6C3F_1$ or BDF_1	ADR or 5-FU

*All drugs were obtained from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

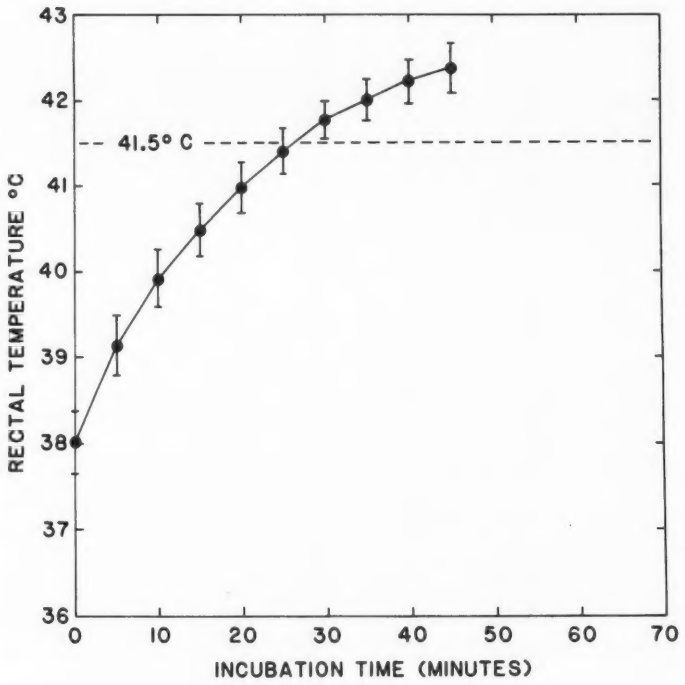

FIGURE 1.—Mean (± SD) rectal temperature response of 48 mice used in as many experimental runs to monitor deep-body temperature during WBH induced at incubator temperatures of 38.8°C–38.9°C. Exposure times were usually 45 mins (occasionally 60 mins).

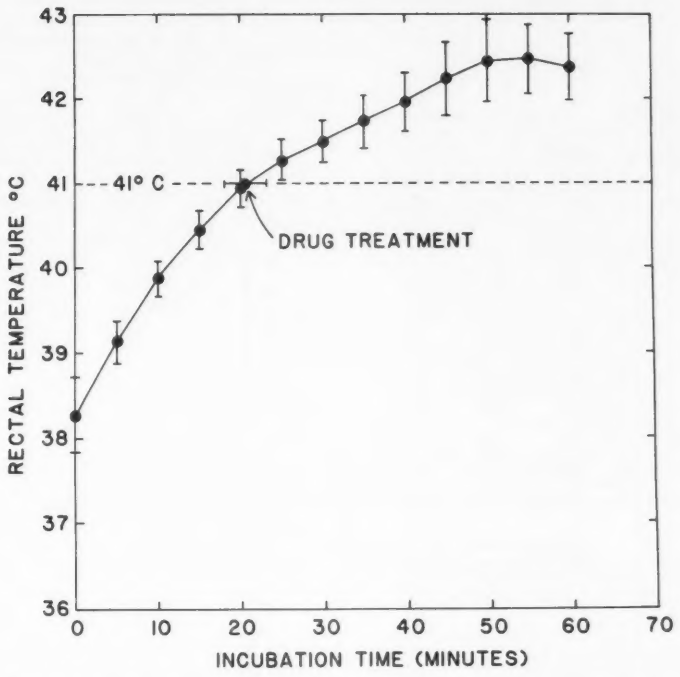

FIGURE 2.—Mean (± SD) rectal temperature response of 12 mice used in as many experimental runs to monitor deep-body temperature during WBH induced at incubator temperatures of 38.8°C–38.9°C. Upon reaching a rectal temperature of 41.0°C, mice received either drug or saline ip. Following injection, incubation was continued for 30 mins.

In unheated mice, the estimated LD10 for CPA was 350 mg/kg, but was reduced to 225 mg/kg in mice receiving WBH. Similarly, the estimated LD10 of MeCCNU in unheated mice was > 41 mg/kg, but was reduced to 28 mg/kg in mice receiving WBH.

VCR given q4days × 3 proved to be more toxic for both BDF_1 and $AKD2F_1$ mice receiving WBH following each drug injection. In unheated BDF_1 mice, 1.3 mg/kg/dose of VCR was the estimated LD10. In heated mice, the LD10 was reduced to about 1 mg/kg/dose. The data for VCR in $AKD2F_1$ mice were obtained from only one experiment, but here too, WBH appeared to increase drug sensitivity.

Differences in drug sensitivities between heated and unheated mice were either minimal or not detected when actinomycin D (act-D), ADR, CCNU, or palmO-ara-C (P-ara-C) was evaluated. In the experiments reported, the lethal toxicity of none of the drugs was decreased by WBH.

Anticancer Activity of Selected Agents Given in Combination With WBH (Rx → WBH)

P388 Leukemia (table 4)

In the eight experiments to be discussed, BDF_1 mice were implanted ip with 10^6 P388 leukemia cells. In each experiment, groups of heated and unheated leukemic control mice receiving drug diluent only were included. The heating regimens consisted of either a single 45-minute (60 minutes in one experiment) heat application on Day 2 or 3 after implant, or three 45-minute heat applications, 4 days apart, beginning on Day 2 after implant. The median survival time (MST) of heated and unheated leukemic mice did not vary by > 2 days in any experiment, and heated mice never had an MST that was > 1 day greater than that of unheated mice, indicating that the degree and duration of WBH used had no likely therapeutic effect (*see* fig 3).

TABLE 3.—Influence of WBH on drug toxicity in mice

Drug	Route	Schedule	Host	Dose (mg/kg/dose)	No. of survivors/total treated			
					Heated mice	Estimated LD10 (mg/kg)	Unheated mice	Estimated LD10 (mg/kg)
ADR	ip	Single dose	BDF_1	15	7/10	10.6	11/30	11
				12	33/40		36/40	
				8	39/40		40/40	
				5.3	29/30		10/10	
	iv	Single dose	$B6C3F_1$	18	—	>15	10/10	>18
				16	—		20/20	
				15	10/10		10/10	
				13	35/35		50/50	
				8.5	34/35		30/30	
				6	33/35		20/20	
			$AKD2F_1$	14	6/10	12.2	9/10	14
				11	10/10		10/10	
				7.5	10/10		10/10	
Act-D	ip	Single dose	BDF_1	1.2	5/20	0.76	11/20	0.77
				1.0	17/20		17/20	
				0.8	33/40		34/40	
				0.5	38/40		39/40	
			$AKD2F_1$	1.2	5/20	0.56	8/20	0.66
				1.0	3/5		5/9	
				0.9	5/10		4/10	
				0.8	15/25		18/30	
				0.7	7/10		6/10	
				0.5	33/35		40/40	
CCNU	ip	Single dose	BDF_1	74	—	46	0/10	47
				57	20/30		22/30	
				39	29/30		29/30	
				26	29/30		10/10	
CPA	ip	Single dose	BDF_1	400	1/10	225	21/30	350
				310	17/35		38/40	
				208	36/39		40/40	
				136	29/30		10/10	
MeCCNU	ip	Single dose	BDF_1	41	17/25	28	28/30	>41
				27	23/25		30/30	
				18	24/25		30/30	
5-FU	ip	q4days × 3	BDF_1	90	3/10	62	13/20	82
				75	5/10		10/10	
				60	19/20		20/20	
				40	8/10		10/10	
			$B6C3F_1$	90	6/14	67	11/20	71
				75	7/14		16/20	
				60	15/15		20/20	
P-ara-C	ip	q4days × 3	$AKD2F_1$	100	3/5	56	1/10	69
				75	13/15		16/20	
				50	12/15		20/20	
				35	10/10		10/10	
VCR	ip	q4days × 3	BDF_1	1.5	14/28	1.05	23/28	1.3
				1.0	27/29		27/28	
				0.7	27/29		28/28	
			$AKD2F_1$	1.2	4/10	ca 0.92	10/10	>1.2
				0.8	10/10		10/10	
				0.5	8/10		10/10	

TABLE 4.—Optimal responses of mice implanted ip with 10^6 P388 leukemia cells to various drugs given with and without WBH (Rx → WBH)*

Experiment No.	Drug (treatment days)	Treatment with WBH* Dose (mg/kg/dose)	No. of survivors/total treated	% ILS (dying mice only)†	Approximate No. of cells alive at end of therapy‡	Treatment without WBH Dose (mg/kg/dose)	No. of survivors/total treated	% ILS (dying mice only)†	Approximate No. of cells alive at end of therapy‡	Conclusion regarding effect of WBH
1	Act-D (2)	0.5	1/10	100	79 (2.3)	0.5	1/10	109	27 (2.3)	No benefit
2	Act-D (2)	1.0	3/10	181	ca 1 (1.2)	0.5	1/10	100	79 (2.3)	Benefit
		0.8	4/1	136	ca 1 (0.9)					
3	Act-D (2)	0.5	1/10	140	3 (2.3)	0.8	0/10	110	79	Benefit
4	Act-D (2)	0.5	2/10	104	16 (1.6)	0.8	0/10	87	1.3×10^2	Benefit
5	ADR (3)	8.0	0/10	130	27	12.0	1/10	150	3 (2.3)	No benefit
6	ADR (3)	8.0	2/10	131	6 (1.6)	8.0	1/10	181	ca 1 (2.3)	No benefit
7	VCR (2, 6, 10)	1.0	0/9	115	5.2×10^3	1.0	1/8	108	1.5×10^4 (2.0)	No benefit
8	VCR (2, 6, 10)	1.0	0/10	150	5.2×10^3	1.0	1/10	160	1.8×10^3 (2.3)	No benefit

*WBH involved heating mice at 38.8°C–38.9°C for 45 mins (60 mins for experiment 3) following drug injection. For a more detailed description of heating regimen, *see* Materials and Methods section.

†Compared to unheated, non-drug-treated, leukemic control mice in the same experiment.

‡Based upon median day of death of dying mice only (26). Nos. in parentheses are based on % survivors (27).

Included in the eight experiments were groups of non-leukemic and leukemic mice that received chemotherapy. In every experiment, several doses of a drug were selected for evaluation based on the approximate LD10 (table 3) of that drug in heated and unheated mice. This approach was used in all the experiments to be discussed in this report. The optimal therapeutic results of the P388 leukemia studies are summarized in table 4.

Actinomycin D.—Mice implanted ip with 10^6 P388 cells were treated on Day 2 after implant with single ip doses of act-D, with or without WBH. All experiments were consistent with historical experience involving treatment of P388 leukemia with act-D, and compared well with each other in terms of their optimal effects and behavior of non-drug-treated leukemic control mice, both heated and unheated. The results of four separate experiments conducted to determine the effect of act-D with and without WBH against P388 leukemia are summarized in figure 3 and table 4. In three of the experiments (Nos. 2–4) minimal therapeutic potentiation was observed in the groups receiving act-D plus WBH based on the difference in the estimated number of cells surviving at the end of therapy (approximately 1 log) and the difference in percent survivors (10%–30%) as compared to groups receiving act-D alone. No beneficial effect was observed with the addition of WBH in one experiment (No. 1) compared to act-D alone. Because of the minimal therapeutic potentiation observed and the lack of complete reproducibility, the value of WBH in combination with act-D against P388 leukemia should be considered equivocal.

Adriamycin.—Two experiments (Nos. 5 and 6) were performed involving the treatment of leukemic mice with single ip doses of ADR on Day 3 after implant of 10^6 P388 cells, with or without WBH (table 4). In both studies, WBH as an adjunctive therapy had no apparent effect on the therapeutic activity of ADR.

Vincristine.—Two experiments (Nos. 7 and 8) were performed in which mice implanted ip with 10^6 P388 leukemia cells were treated with VCR, ip q4days × 3, beginning on Day 2 after implant; WBH, when given in combination with VCR, was employed after each injection (table 4). In both studies, multiple WBH exposures provided no additional benefit to leukemic mice receiving VCR.

Lewis Lung Carcinoma (LL) (table 5)

Data from eight experiments (Nos. 9–16) in which mice were implanted sc with LL and treated with WBH, with or without drug treatment, are shown in table 5. The mortality rates of mice given WBH (no drug) or maintained at ambient temperature (no drug) were compared (data not shown). The pooled MST in both groups (39–40 mice each) was 30 days and in no instance did LL-bearing mice receiving WBH have an MST that was > 21% greater than the parallel group of LL-bearing mice not given WBH. Thus, WBH alone, in the manner employed (a single, 45-minute incubation period [except experiment No. 13, where a 60-minute incubation period was used] given 2, 6, or 7 days after implant), did not influence the mortality rate of mice bearing sc implanted LL.

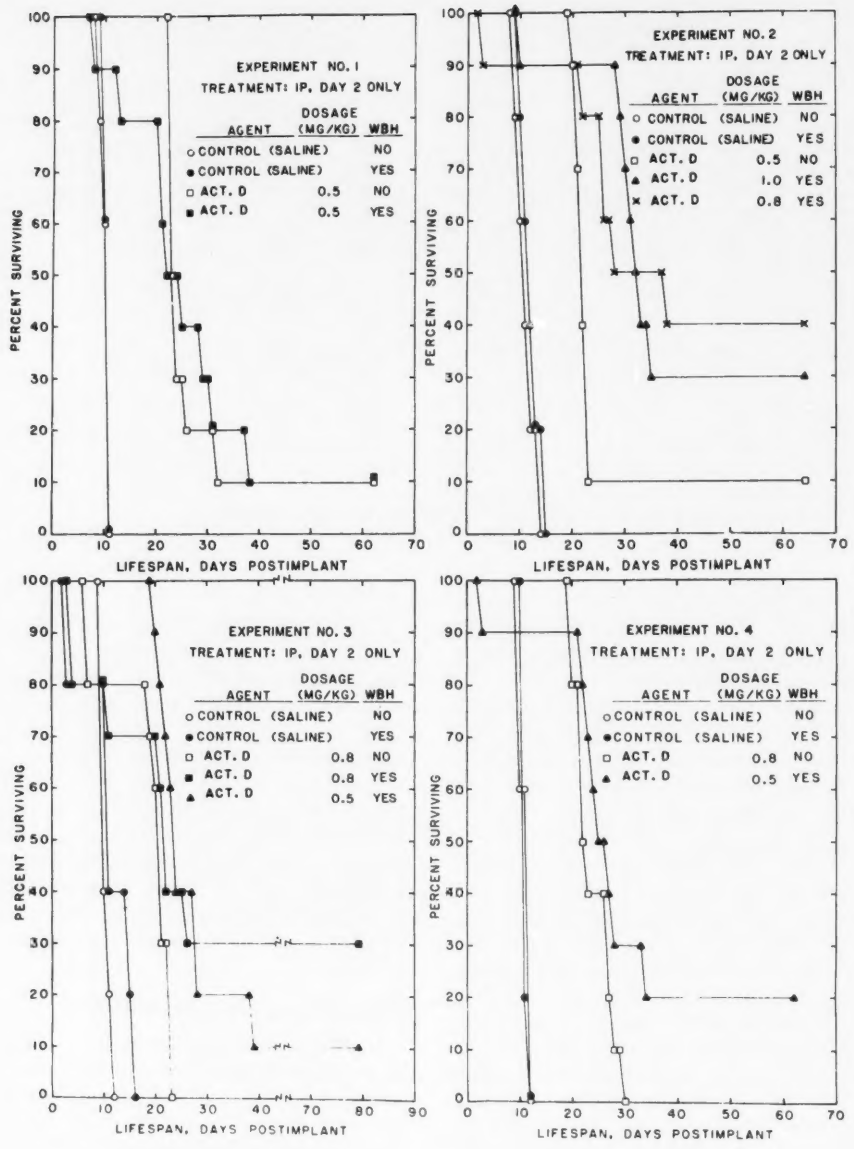

FIGURE 3.—Therapeutic activity of act-D alone or in combination with WBH (Rx → WBH) in mice implanted ip with 10^6 P388 leukemia cells.

TABLE 5.—Optimal responses of mice bearing solid tumors to various drugs given with and without WBH (Rx → WBH)*

Experiment No.	Tumor	Drug (treatment days)	Treatment with WBH* Dose (mg/kg/dose)	No. of complete regressions/ total treated†	No. of survivors/ total treated	% ILS (dying mice only)‡	Treatment without WBH Dose (mg/kg/dose)	No. of complete regressions/ total treated†	No. of survivors/ total treated	% ILS (dying mice only)‡	Conclusion regarding effect of WBH
9	Lewis lung carcinoma	MeCCNU (7)	27	0/10	0/10	22	41	3/10	3/10	3	No benefit
10		MeCCNU (7)	36	0/10	0/10	19	41	2/10	2/10	25	No benefit
11		CCNU (6)	39	2/10	2/10	25	39	0/10	0/10	–4	Benefit
			26	1/10	1/10	50					
12		CCNU (6)	39	0/10	0/10	7	57	1/10	1/10	36	No benefit
13		CCNU (6)	39	0/10	0/10	11	57	0/10	0/10	–10	No benefit
14		CCNU (6)	39	0/10	0/10	29	57	1/10	0/10	14	No benefit
15		CPA (2)	136	—§	8/10	1	310	—§	10/10	—	No benefit
16		CPA (2)	208	—§	6/10	67	208	—§	7/10	100	No benefit
17	Mammary adenocarcinoma 16C	ADR (14)	13	7/10	0/10	17	16	9/10	0/10	29	No benefit
18		ADR (12)	15	1/10	0/10	52	18	2/10	0/10	72	No benefit
19	Colon carcinoma 38	ADR (12)	13	6/10	3/10	84	13	6/10	5/10	56	No benefit
20		5-FU (19, 23, 27)	60	0/10	0/10	92	60	0/10	0/10	41	Inconclusive
21		5-FU (19, 23, 27)	60	0/10	0/10	9	60	0/10	0/10	19	No benefit
22	Ridgway osteogenic sarcoma	Act-D (18)	0.5	2/5	1/5	69	0.5	1/10	0/10	66	Possible benefit
23		Act-D (18)	0.5	4/10	1/10	70	0.5	2/10	0/10	85	
24		Act-D (18)	0.7	4/10	3/10	119 (LD20)	0.7	7/10	5/10	129	No benefit
25		P-ara-C (12,	75	6/10	0/10	106	75	9/9	1/9	139	No benefit

*WBH involved heating the mice at 38.8°C–38.9°C for 45 mins (60 mins for experiment 13) following drug injection. For a more detailed description of heating regimen, *see* Materials and Methods section.

†Complete regression of tumor below palpable level (about 65 mg).

‡Compared to unheated, non-drug-treated, tumor control mice in the same experiment.

§Tumors not advanced enough at time of treatment to evaluate for complete regressions.

Methyl-CCNU.—This drug was evaluated alone and in combination with WBH against sc implanted LL. Drug treatment (with or without WBH) was given as a single ip dose on Day 7 after implant at which time the tumors averaged about 0.5 g. It has been reported that LL at this size and time after implant will have produced essentially 100% metastasis to the lungs (30). No beneficial effect due to WBH was observed in two separate experiments (Nos. 9 and 10) when MeCCNU was given in combination with WBH (table 5).

CCNU.—Four experiments (Nos. 11–14) were performed in which LL-bearing mice were treated on Day 6 after implant with single ip doses of CCNU alone and in combination with WBH. In experiment No. 11, in the groups treated with 39 or 26 mg/kg of CCNU in combination with WBH, there were two and one complete regressions among ten animals each with 25% and 50% ILS respectively, compared to no complete regressions among ten animals and no increase in lifespan in the group treated with 39 mg/kg of CCNU alone. Three additional experiments (Nos. 12–14) were performed in an attempt to confirm the preceding observation. No beneficial effect was obtained with the addition of WBH compared to CCNU alone. The overall data suggest that the addition of WBH to CCNU therapy for LL probably did not add to the therapeutic effectiveness of CCNU without WBH.

Cyclophosphamide.—Two experiments (Nos. 15 and 16) were performed in which mice bearing sc implanted LL were treated with single ip doses of CPA on Day 2 after implant with or without WBH. Considering the high cure rates obtained using CPA alone in these studies, a more advanced stage of the disease prior to chemotherapy would have allowed a better assessment of the efficacy of adjunctive WBH. Nevertheless, in both experiments, treatment with WBH plus CPA resulted in therapeutic responses that were, at best, only comparable to those achieved using CPA alone.

Mammary Adenocarcinoma 16C (table 5)

Two experiments (Nos. 17 and 18) were done in which the effects of ADR alone and in combination with WBH against sc implanted MAM 16C in $B6C3F_1$ mice were determined. No beneficial effect was obtained with the addition of WBH compared to ADR alone. MAM 16C was essentially unresponsive to WBH alone (no drug), as the MST of mice given a single exposure of WBH (12 or 14 days after implant; tumor size, 400 mg) was 36 days compared to 31.5 days in unheated tumor control mice (ten mice/group, data not shown).

Colon Carcinoma 38 (CC 38) (table 5)

Three experiments were done in which the effects of ADR or 5-FU alone and in combination with WBH against advanced (≥ 400 mg) sc implanted CC 38 in either BDF_1 or $B6C3F_1$ mice were determined. WBH alone (no drug) did not affect the mortality rate among mice bearing CC 38. The MST of mice given WBH was 62 days compared to 60.5 days in unheated tumor control mice (19–20 mice/group, data not shown).

Adriamycin.—The optimal responses obtained with ADR alone and in combination with WBH against CC 38 were essentially the same based on number of complete regressions, survivors, and percent increase in lifespan (% ILS) (experiment No. 19). Thus, under the conditions of this study, the addition of WBH did not improve the optimal therapeutic response achieved with ADR alone.

5-Fluorouracil.—Based on % ILS, the addition of WBH to 5-FU therapy for CC 38 (experiment No. 20) produced a beneficial effect over that obtained with 5-FU alone (92% ILS obtained with 5-FU plus WBH as compared to 41% ILS obtained with 5-FU alone). However, the tumor growth patterns (data not shown) of both treatment groups were very similar. Thus, even though 5-FU plus WBH produced more than double the % ILS achieved with 5-FU alone, the therapeutic value of the addition of WBH must be considered equivocal. In a repeat experiment (No. 21), neither 5-FU nor 5-FU plus WBH proved to be effective against CC 38 at acceptable levels of toxicity (≤ LD10).

Ridgway Osteogenic Sarcoma (ROS) (table 5)

It was difficult to acquire valid data involving ROS because of the greater susceptibility of $AKD2F_1$ mice (compared to the other hybrid strains) to the heating regimen used. Many of the drug treatments for mice bearing ROS involved multiple injection schedules, and the administration of WBH following each drug injection only compounded the problem. Based upon data from three experiments, WBH alone had no effect on the mortality rate following sc implantation of ROS. The MST of 15 mice receiving either single 45-minute exposures of WBH on Day 18 after implant (tumor size, 2–3 g) or 45-minute exposures on Days 12, 16, and 20 after implant (tumor size, 0.5–1.0 g on Day 12) was 36 days. In comparison, 15 mice (from these three experiments) that did not receive WBH had a similar MST of 31 days. Data from approximately 160 ROS experiments conducted in this laboratory indicate that this 5-day difference in MST between those receiving and not receiving WBH is within the normal range of lifespan of ROS-bearing (non-drug-treated) control mice.

A summary of the optimal therapeutic responses achieved among ROS-bearing mice receiving drug therapy, with or without WBH, is presented in table 5.

Actinomycin D.—Data from three experiments in which mice bearing advanced (2–3 g) ROS tumors were treated with act-D with and without WBH are shown in table 5. In two experiments (Nos. 22 and 23) the addition of WBH to act-D treatment resulted in 40% complete tumor regression as compared to 10%–20% complete tumor regression in the groups treated with act-D alone. However, the % ILS among dying mice was not increased with the addition of WBH. In experiment No. 24, no benefit based on complete tumor regression rate or % ILS was obtained with WBH plus act-D compared to act-D alone. These data suggest that mice bearing ROS did not benefit from the combination of WBH plus act-D when compared with act-D therapy alone.

PalmO-ara-C.—When administered on a q4days × 3 schedule to mice implanted sc 12 days earlier with ROS, P-ara-C caused nine of nine unheated mice to have complete tumor regression (no cures) and achieve a 139% ILS, and caused six of ten heated mice to have complete tumor regression (no cures) and achieve a 106% ILS. Data from this single unconfirmed experiment indicate that mice bearing ROS did not benefit from being subjected to WBH following each treatment with P-ara-C.

Anticancer Activity of Selected Agents Given Simultaneously With WBH (Rx During WBH)

Six experiments were performed in which mice were treated with selected agents simultaneously with WBH. The details of this method of administering WBH are described in the Materials and Methods section. Briefly, the major change with this method was that the mice were brought to a hyperthermic state (41.0°C) and then given drug in an attempt to have hyperthermia coincide temporally with maximum blood concentrations of drugs. The drug-tumor systems chosen for evaluation included those where occasional suggestions of therapeutic potentiation between drug and WBH were found using drug treatment followed by induction of WBH.

P388 Leukemia (figs 4 and 5)

Actinomycin D.—Two experiments (Nos. 26 and 27) were performed in which BDF_1 mice implanted ip with 10^6 P388 cells were treated on Day 2 after implant with

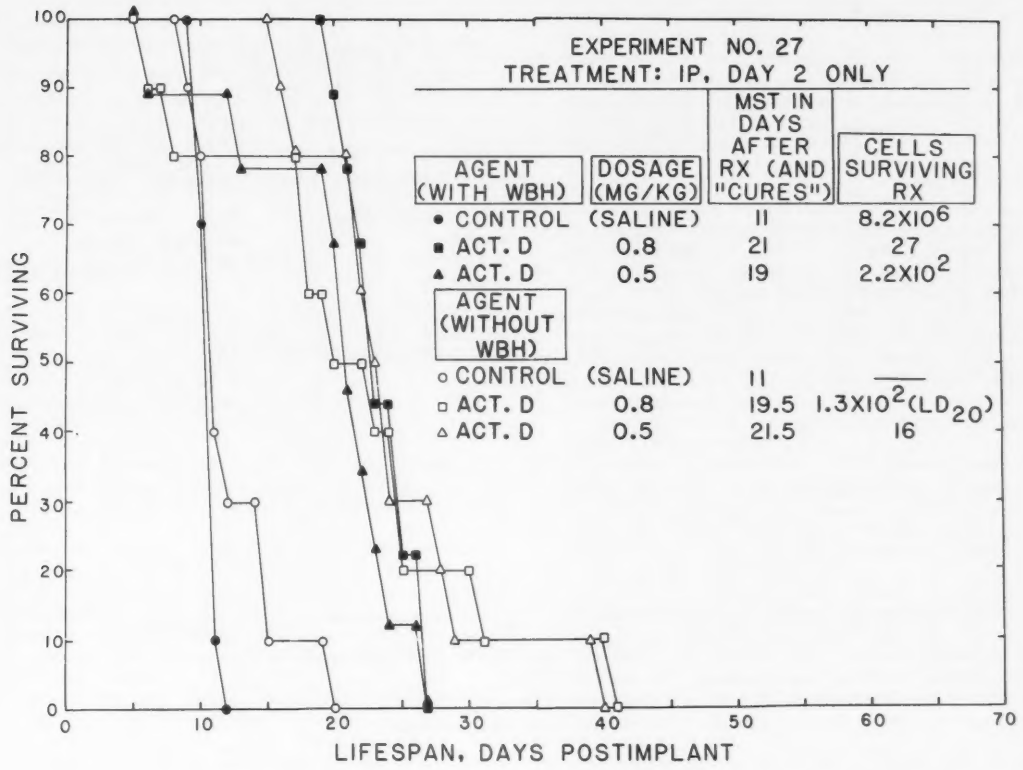

FIGURE 4.—Therapeutic activity of act-D alone or in combination with WBH (Rx during WBH) in mice implanted ip with 10^6 P388 leukemia cells.

single ip doses of act-D, with or without WBH. In experiment No. 26 (data not shown), WBH alone had no effect on P388-induced mortality (consistent with all our previous WBH studies). A dose of 0.8 mg/kg of act-D, although an LD10 in nonleukemic mice in the presence or absence of heat, appeared to have been ≥ LD20 in heated and unheated leukemic mice. Nevertheless, at this dose, or the next lower dose, 0.5 mg/kg, act-D given during WBH was not therapeutically superior to act-D alone. In experiment No. 27 (fig 4), WBH alone again had no effect on P388-induced mortality. Act-D alone, 0.5 mg/kg, cured none of the leukemic mice but did reduce the leukemic cell population surviving treatment to approximately 16 cells/mouse. Act-D plus WBH similarly failed to cure any mice but did reduce the leukemic cell population to approximately 27 cells/mouse. Thus, based on these two experiments, act-D given during WBH was not therapeutically superior to act-D alone in treating mice bearing P388 leukemia.

Adriamycin.—Two experiments (Nos. 28 and 29) were performed in which single ip doses of ADR, with or without WBH, were administered to mice 2 days following implantation of 10^6 P388 leukemia cells. In neither experiment did WBH alone have any effect on P388-induced mortality. In experiment No. 28 (data not shown), among heated leukemic mice, ADR (9 or 12 mg/kg) caused three of ten mice to die in advance of leukemic control mice and these deaths were therefore considered to be treatment-associated (in parallel nonleukemic heat-treated drug toxicity control mice, 12 mg/kg of ADR caused only one death among ten animals). Excluding those mice that died early (in an attempt to salvage this experiment for the purpose of providing some data for evaluation), the optimal response rate for mice treated with ADR plus WBH was one of seven mice cured and (an adjusted) ILS of 260% or about one leukemic cell/mouse surviving therapy, on the average, for the entire group. This response was not superior to the two of ten mice cured and 240% ILS achieved using ADR alone. In experiment No. 29 (fig 5), ADR alone, 9 mg/kg, cured seven of ten mice. ADR, 9 mg/kg, plus WBH may again have been an LD30 (it was the lowest dose tested in combination with WBH) and

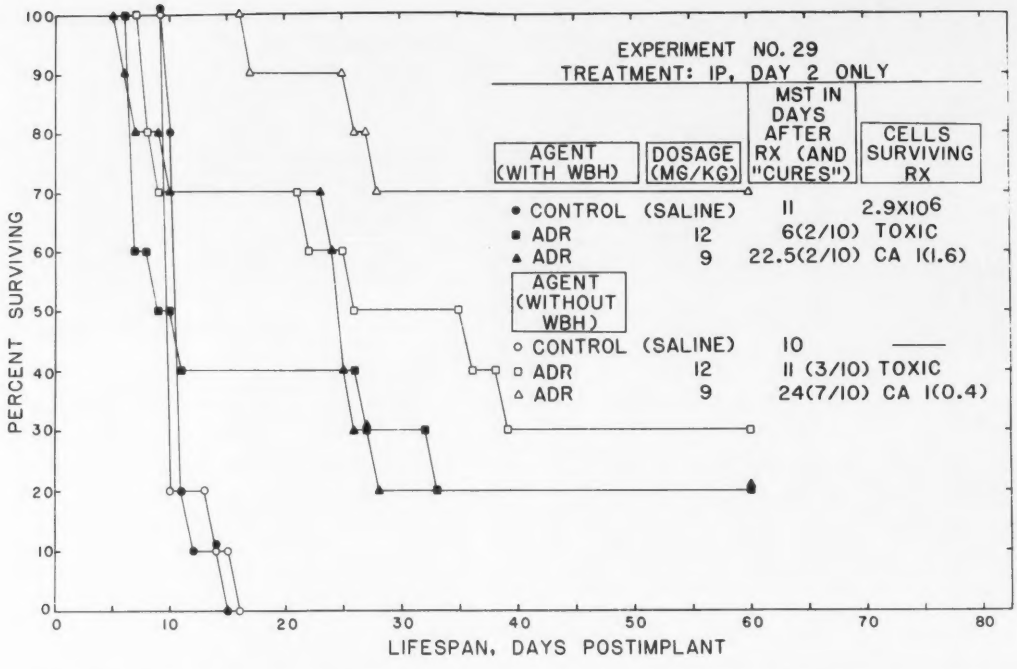

FIGURE 5.—Therapeutic activity of ADR alone or in combination with WBH (Rx during WBH) in mice implanted ip with 10^6 P388 leukemia cells.

only cured two of ten mice. Thus, as evaluated, WBH did not provide additional therapeutic benefit to mice bearing P388 leukemia and treated with ADR.

Lewis Lung Carcinoma

CCNU.—Two experiments were done in which mice bearing sc implanted LL tumors were treated with single ip doses of CCNU only or during WBH on Day 6 after implant when the tumors were about 300–400 mg. WBH alone had no effect on the growth of LL or the mortality rate (data not shown). In the first experiment, CCNU alone caused no partial regressions and an optimal response of only 36% ILS. CCNU given during WBH caused one partial regression among ten animals and a 7% ILS. In the second experiment, neither CCNU alone nor CCNU given during WBH produced a positive % ILS compared to untreated tumor-bearing mice. Thus, WBH failed to provide any additional therapeutic benefit to mice bearing LL and treated with CCNU.

DISCUSSION

During the past few years, there has been a renewal of interest in the use of hyperthermia for the treatment of cancer. This is reflected by many experimental reports, some of which have been summarized in table 1 (8–22), and several clinical studies in which hyperthermia has been used (eg, 1,2,23–25). Hyperthermia has a selective destructive effect on cancer cells (1–7), the lethal temperature range beginning at 41.5°C (31). The operational range for WBH in man and animals is 41.5°C–42.0°C; "although more rapid destructive effects are achieved at intratumor temperatures in excess of 42.0 C . . . host tolerance considerations preclude the use of these" (31).

Although we were aware that small laboratory animals may make poor model systems for WBH investigations (31), we proceeded to evaluate the efficacy of WBH, alone and in combination with chemotherapy, for the treatment of mice bearing several different drug-sensitive tumors. We felt that neither local hyperthermia nor surgery addressed the problem of disseminated neoplastic disease and that WBH may have been able to provide some benefit under such conditions. Several months were spent establishing the limits of temperature exposure tolerated by different strains of mice of both sexes at various ages. We settled upon the first heating regimen (Rx → WBH) described in the Materials and Methods section and eventually modified the protocol to that described as the second heating method (Rx during WBH). These heating regimens may justly be criticized for not providing sufficient WBH, either in terms of temperature or duration, necessary to favorably influence the outcome of therapy. Yet we were guided by the desire not to stress the mice

beyond LD10 levels and we were indeed successful in keeping treatment-associated mortality to within acceptable boundaries by using the WBH protocols described. Based on the data presented and with the methods employed, a consistent, convincing therapeutic benefit associated with the use of WBH as an adjunct to the chemotherapeutic treatment of murine neoplasms was not apparent.

REFERENCES

1. Selective heat sensitivity of cancer cells. *In* Recent Results in Cancer Research (Rossi-Fanelli A, Cavaliere R, Mondovi B, et al, eds). Berlin, Germany, Springer-Verlag, 1977, vol 59.
2. Cavaliere R, Ciocatto EC, Giovanella BD, et al. Selective heat sensitivity of cancer cells. Cancer 20:1351–1381, 1967.
3. Mondovi B, Strom R, Rotilio G, et al. The biochemical mechanism of selective heat sensitivity of cancer cells. I. Studies on cellular respiration. Eur J Cancer 5:129–136, 1969.
4. Mondovi B, Agro AF, Rotilio G, et al. The biochemical mechanism of selective heat sensitivity of cancer cells. II. Studies on nucleic acids and protein synthesis. Eur J Cancer 5:137–146, 1969.
5. Kase K, and Hahn GM. Differential heat response of normal and transformed human cells in tissue culture. Nature 255:228–230, 1975.
6. Turano C, Ferraro A, Strom R, et al. The biochemical mechanism of selective heat sensitivity of cancer cells. III. Studies on lysosomes. Eur J Cancer 6:67–72, 1970.
7. Overgaard J. Ultrastructure of a murine mammary carcinoma exposed to hyperthermia in vivo. Cancer Res 36:983–995, 1976.
8. Dickson JA, and Ellis HA. The influence of tumor volume and the degree of heating on the response of the solid Yoshida sarcoma to hyperthermia (40–42°). Cancer Res 36:1188–1195, 1976.
9. Dickson JA, and Suzangar M. In vitro-in vivo studies on the susceptibility of the solid Yoshida sarcoma to drugs and hyperthermia (42°). Cancer Res 34:1263–1274, 1974.
10. Dickson JA, and Muckle DS. Total-body hyperthermia versus primary tumor hyperthermia in the treatment of the rabbit VX-2 carcinoma. Cancer Res 32:1916–1923, 1972.
11. Mendecki J, Friedenthal E, and Botstein C. Effects of microwave-induced local hyperthermia on mammary adenocarcinoma in C3H mice. Cancer Res 36:2113–2114, 1976.
12. Thrall DE, Gillette EL, and Bauman CL. Effect of heat on the C3H mouse mammary adenocarcinoma evaluated in terms of tumor growth. Eur J Cancer 9:871–875, 1973.
13. Thrall DE, Gillette EL, and Dewey WC. Effect of heat and ionizing radiation on normal and neoplastic tissue of the C3H mouse. Radiat Res 63:363–377, 1975.
14. Overgaard J. Combined Adriamycin and hyperthermia treatment of a murine mammary carcinoma in vivo. Cancer Res 36:3077–3081, 1976.
15. Hahn GM, Braun J, and Har-Kedar I. Thermochemotherapy: synergism between hyperthermia (42–43°) and Adriamycin (or bleomycin) in mammalian cell inactivation. Proc Natl Acad Sci USA 72:937–940, 1975.
16. Bleehen NM, Honess DJ, and Morgan JE. Interaction of hyperthermia and the hypoxic cell sensitizer Ro-07-0582 on the EMT6 mouse tumor. Br J Cancer 35:299–306, 1977.
17. Marmor JB, Hahn N, and Hahn GM. Tumor cure and cell survival after localized radiofrequency heating. Cancer Res 37:879–883, 1977.
18. Hahn GM, Li GC, and Shiu E. Interaction of amphotericin B and 43° hyperthermia. Cancer Res 37:761–764, 1977.
19. Twentyman PR, Morgan JE, and Donaldson J. Enhancement by hyperthermia of the effect of BCNU against the EMT6 mouse tumor. Cancer Treat Rep 62:439–443, 1978.
20. Alfieri AA, Hahn EW, and Kim JH. The relationship between the time of fractionated and single doses of radiation and hyperthermia on the sensitization of an in vivo mouse tumor. Cancer 36:893–903, 1975.
21. Yerushalmi A. Treatment of a solid tumor by local simultaneous hyperthermia and ionizing radiation: dependence on temperature and dose. Eur J Cancer 12:807–813, 1976.
22. Hofer KG, Choppin DA, and Hofer MG. Effect of hyperthermia on the radiosensitivity of normal and malignant cells in mice. Cancer 38:279–287, 1976.
23. Stehlin JS, Jr, Giovanella BC, Opolyi PD, et al. Results of hyperthermic perfusion for melanoma of the extremities. Surg Gynecol Obstet 140:339–348, 1975.
24. Hall RR, Schade ROK, and Swinney J. Effects of hyperthermia on bladder cancer. Br Med J 2:593–594, 1974.
25. Pettigrew RT, Galt JM, Ludgate CM, et al. Clinical effects of whole-body hyperthermia in advanced malignancy. Br Med J 4:679–682, 1974.
26. Schabel FM, Jr, Griswold DP, Jr, Laster WR, Jr, et al. Quantitative evaluation of anticancer agent activity in experimental animals. Pharmacol Ther [A]1:411–436, 1977.
27. Skipper HE, Schabel FM, Jr, and Wilcox WS. Experimental evaluation of potential anticancer agents. XIII. On the criteria and kinetics associated with "curability" of experimental leukemia. Cancer Chemother Rep 15:1–111, 1964.
28. Corbett TH, Griswold DP, Jr, Roberts BJ, et al. Evaluation of single agents and combinations of chemotherapeutic agents in mouse colon carcinomas. Cancer 40:2660–2680, 1977.
29. Corbett TH, Griswold DP, Jr, Roberts BJ, et al. Biology and therapeutic response of a mouse mammary adenocarcinoma (16/C) and its potential as a model for surgical adjuvant chemotherapy. Cancer Treat Rep 62:1471–1488, 1978.
30. Mayo JG, Laster WR, Jr, Andrews CM, et al. Success and failure in the treatment of solid tumors. III. "Cure" of metastatic Lewis lung carcinoma with methyl-CCNU (NSC-95441) and surgery-chemotherapy. Cancer Chemother Rep 56:183–195, 1972.
31. Dickson JA. Letter: Hyperthermia in the treatment of cancer. Cancer Chemother Rep 58:294–296, 1974.

Normal Metyrapone Response After 1 Month of High-Dose Methylprednisolone in Cancer Patients: A Phase I Study [1,2,3]

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SUMMARY

A dose of 125 mg of methylprednisolone was given iv each morning for 28 days to six patients with cancer. The posttreatment mean AM/PM serum cortisol (hydrocortisone) level of 19.55/11.27 μg/dl showed a statistically significant ($P < 0.05$) diurnal rhythm and did not differ from the pretreatment level (19.28/11.85 μg/dl). The response to metyrapone (750 mg orally, every 4 hours × six doses) was assessed in five of the six patients before and after treatment. No abnormally low Compound S (11-deoxycortisol) levels were observed. The mean serum Compound S level (15.30 μg/dl) achieved after treatment did not differ significantly from the pretreatment mean level (20.24 μg/dl, $P > 0.1$). No adverse clinical effects were observed during or after this steroid treatment. Since significant hypothalamic-pituitary-adrenal suppression has not been demonstrated, this regimen appears to be safe for use in controlled studies of clinical efficacy.

[Cancer Treat Rep 63:1327–1330, 1979]

Synthetic glucocorticoids, primarily prednisone, are currently used with antineoplastic intent in combination chemotherapy for various neoplasms, including breast cancer and Hodgkin's disease (1,2). They are also frequently used in a somewhat different group of patients with a variety of malignancies where the primary objective is palliative. Numerous studies have suggested that symptoms such as anorexia, weight loss, dysphagia, and dyspnea may be ameliorated (3,4). Unfortunately, well-defined efficacy has not been established in these circumstances because most of these studies have been uncontrolled, the quantitation of palliation is extremely difficult, and a systematized approach to drug and schedule selection has not been applied.

This report describes a phase I study designed to test the safety of a 1-month, high-dose, once daily regimen of methylprednisolone (Solu-Medrol) (6-α-MP) with regard to hypothalamic-pituitary-adrenal (HPA) suppression. It was reasoned that if provocative testing could show the HPA axis to be unaffected, then subjects would not require a "tapering" period after therapy.

METHODS

Six cancer patients, including two females and four males ranging in age from 61 to 85 years (mean, 70.3 years), were studied. Three patients had adenocarcinoma of the colon, two had adenocarcinoma of the prostate, and one had lymphoma; all patients had symptoms such as pain and anorexia no longer treatable by conventional antineoplastic therapy. All were inpatients at the Winnipeg Municipal Hospital, an extended-care institution. Patients were excluded if they had received corticosteroids within the preceding month, had an anticipated survival of < 1 month, were suspected of having a bacterial infection, had a recent history of herpes zoster or peptic ulcer, or were receiving drugs which would interfere with plasma corticosteroid determinations. Informed consent was obtained from all patients. Serum cortisol (hydrocortisone) and Compound S (11-deoxycortisol) determi-

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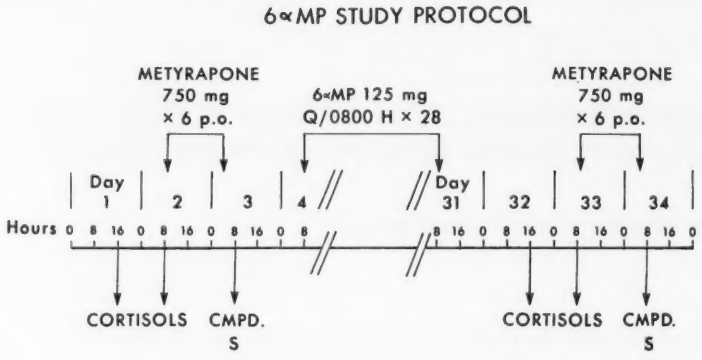

FIGURE 1.—Diagram of treatment protocol. The timing of metyrapone administration, corticosteroid determinations, and 6-α-MP administration are shown on separate time scales separated by diagonal breaks. CMPD. S = Compound S.

nations were done by a radioimmunoassay technique (5,6). In order to avoid interassay variability, all samples for each subject were analyzed in the same assay run.

The treatment and testing protocol is illustrated in figure 1. First, afternoon (1600 hours) and morning (0800 hours) cortisol levels were obtained, and a baseline Compound S level was determined. Second, 750 mg of metyrapone was administered every 4 hours orally × six doses; 4 hours after the last dose, Compound S was again determined. This constituted a "complete metyrapone challenge." Third, 125 mg of 6-α-MP was first given iv at 0800 hours, the day after the last dose of metyrapone; it was continued daily at the same hour for a total of 28 doses. Fourth, allowing a 1-day interval (Day 32, fig 1) after the completion of 6-α-MP treatment, baseline PM and AM cortisol levels were again obtained. Finally, the complete metyrapone challenge was repeated. Corresponding AM and PM values and pretreatment and posttreatment values were compared by paired-T analysis and the differences were considered significant if P was < 0.05.

RESULTS

Table 1 shows the serum cortisol determinations before and after 6-α-MP administration. Table 2 shows the serum Compound S and cortisol response to metyrapone before and after 6-α-MP treatment. Patient 6 could not be studied with the second metyrapone challenge because of an intervening acute upper respiratory infection.

There was a significant diurnal variation of serum cortisol levels (AM values higher than PM values) both before and after 6-α-MP ($P < 0.05$). Mean cortisol levels at both times of the day did not differ significantly (posttreatment versus pretreatment 6-α-MP, $P > 0.1$). All metyrapone responses both before and after 6-α-MP therapy were within normal limits as defined by Spark (7), ie, the premetyrapone serum Compound S levels (not shown) were all 1 μg/dl, the serum cortisol levels (table 2) all declined to < 40% of the serum Compound S value (indicating an effective block of 11-β-hydroxylase), and the serum Compound S response was > 10.5 μg/dl. Although

TABLE 1.—Diurnal variation of serum cortisol (μg/dl) before and after a 28-day course of 6-α-MP

Patient No.	Serum cortisol level (μg/dl)—			
	Before 6-α-MP		After 6-α-MP	
	AM	PM	AM	PM
1	17.4	2.2	15.5	6.8
2	28.5	20.9	22.6	8.5
3	21.8	16.5	18.0	7.9
4	18.5	13.3	25.3	24.5
5	19.7	12.3	19.6	8.0
6	9.8	5.9	16.3	11.9
Mean	19.28	11.85	19.55	11.27

TABLE 2.—Postmetyrapone serum Compound S and cortisol levels before and after a 28-day course of 6-α-MP

	Serum level (μg/dl) of—			
	Compound S		Cortisol	
Patient No.	Before 6-α-MP	After 6-α-MP	Before 6-α-MP	After 6-α-MP
1	13.1	15.1	1.1	1.0
2	28.2	20.6	1.0	4.3
3	22.0	14.7	5.5	4.2
4	26.2	19.8	1.0	2.8
5	11.7	10.8	3.7	4.3
6	21.6*	—	1.0*	—
Mean	20.24	15.30	3.88	3.32

*Not included in analysis since 2nd metyrapone challenge was incomplete (*see* text for details).

Compound S values after 6-α-MP were lower than values before 6-α-MP in four of five subjects assessed, the differences were small and overall there was no statistically significant difference between mean values ($P > 0.1$). Clinically, no adverse effects including overt signs of hypercortisolism were detected either during 6-α-MP administration for 28 days or after its discontinuation.

DISCUSSION

In this study, a 1-month administration of 6-α-MP was shown to be safe as judged by retention of normal diurnal cortisol rhythm, normal HPA responses to metyrapone challenge, and lack of "withdrawal symptoms" (anorexia, nausea, emesis, lethargy, headache, fever, desquamation, arthralgia, myalgia, weight loss, and postural hypotension) following discontinuation of administration. That some "blunting" of the metyrapone response may be present, however, is suggested by the observation that four of the five patients studied demonstrated a lesser, but still normal, response to metyrapone after the 6-α-MP treatment period.

Although there does not appear to be general agreement (8,9) with regard to the best mode of testing for HPA suppression following glucocorticoid therapy, Axelrod (9) suggests that the metyrapone test may be the best available indicator of HPA function which does not require a stressful challenge. Using this test, abnormalities in the HPA system have been observed as early as 3 days after starting therapy with cortisol (100 mg/day). It is therefore concluded that the present treatment regimen produces no clinically significant HPA suppression.

6-α-MP was selected for study because it was available in parenteral form; the iv route was desirable to minimize gastrointestinal intolerance and to circumvent the possibility of variable absorption by the oral route. Its plasma half-life (88–178 minutes) is probably comparable to that of prednisone, although the data are scant (9). Furthermore, according to Axelrod, the glucocorticoid potency of 6-α-MP is the highest of any commonly available "short-acting" glucocorticoid and its mineralocorticoid potency is minimal. The early morning time of administration was chosen to minimize perturbation of the endogenous diurnal rhythm and thus reduce the possibility of HPA suppression (9). The dose of 125 mg was selected because the drug is commercially available in this quantity, because, in equivalent glucocorticoid activity, it exceeds the potency of most currently used prednisone regimens, and because its use has been described in the European literature (4).

In conclusion, we were surprised that no significant HPA suppression was observed with such high doses of glucocorticoid administered over 1 month. This is in contrast to the generally accepted practice of not exceeding 2 weeks' therapy with intermittent antineoplastic prednisone regimens. The 1-month regimen may now be used in definitive controlled studies of both specific antineoplastic and palliative effects in medical oncology without concern for significant HPA suppression or the necessity for dose tapering.

REFERENCES

1. ROSSI A, BRAMBILLA C, and BONADONNA G. Controlled study with l-phenylalanine mustard (L-PAM) and 5-fluorouracil (5-FU) plus or minus prednisone in advanced breast cancer. Tumori 62:193–196, 1976.
2. DEVITA VT, JR, SERPICK AA, and CARBONE PP. Combination chemotherapy in the treatment of advanced Hodgkin's disease. Ann Intern Med 73:881–895, 1970.
3. SCHELL HW. Adrenal corticosteroid therapy in far-advanced cancer. Geriatrics 27:131–141, 1972.
4. POMMATAU E, REVILLARD JP, and GIGNOUX M. Importance of sodium hemisuccinate of methyl-6-prednisolone (Solu Medrol) in the treatment of malignant tumors. Dimanche 49:1179–1198, 1962.
5. CARTER JN, TYSON JE, WARNE GL, ET AL. Adrenocortical function in hyperprolactinemic women. J Clin Endocrinol Metab 45:973–980, 1977.
6. MAHAJAN DK, WHALEN JD, TYLER FH, ET AL. Plasma 11-deoxycortisol radioimmunoassay for metyrapone tests. Steroids 20:609–620, 1972.

7. SPARK RF. Simplified assessment of pituitary-adrenal reserve. Measurement of serum 11-deoxycortisol and cortisol after metyrapone. Ann Intern Med 75:717–723, 1971.
8. KOCH-WESER J. Withdrawal from glucocorticoid therapy. N Engl J Med 295:30–32, 1976.
9. AXELROD L. Glucocorticoid therapy. Medicine 55:39–65, 1976.

Evaluation of Cyclocytidine in Children With Advanced Acute Leukemia and Solid Tumors [1]

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SUMMARY

Cyclocytidine, a slow-release form of cytosine arabinoside, was evaluated in 69 children with advanced acute leukemia and solid tumors. One child with acute lymphocytic leukemia attained a complete remission. This child had received intrathecal cytosine arabinoside prior to the cyclocytidine. Eighteen of the 31 patients with acute lymphocytic leukemia/acute undifferentiated leukemia who did not respond received two or more courses of the drug. There were no responses in 15 children with acute myelogenous leukemia, in 11 children with neuroblastoma, or in 11 children with various solid tumors of childhood. A dose of 600 mg/m^2/day for 10 consecutive days is tolerated in children.

[Cancer Treat Rep 63:1331–1333, 1979]

Cyclocytidine is of interest because it is a slow-release form of cytosine arabinoside (ara-C) (1,2). Therefore, the need to utilize continuous infusions of ara-C would possibly be obviated if the clinical activity of cyclocytidine and ara-C were similar. Cyclocytidine is resistant to deamination by pyrimidine nucleoside deaminase, but is hydrolyzed to ara-C and then follows standard conversion to the phosphorylated derivative and deamination to uracil arabinoside.

Cyclocytidine has exhibited activity against a variety of mouse tumor systems, including L1210 leukemia, the lymphocytic sarcoma of Nakahara and Tokuzen (3), and the murine neuroblastoma model (4). The drug exerts its cytotoxic effect through interference with DNA synthe-

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[2] This study was done by the Children's Cancer Study Group. The following institutions (with investigators and grant support) participated in this study: Children's Cancer Study Group Operations Office, University of Southern California Comprehensive Cancer Center, Los Angeles (*D. Hammond, J. Weiner, R. Honour, H. Sather,* and *J. Anderson,* CA-13539); University of Michigan Medical Center, Ann Arbor (*R. Heyn,* CA-02971); Children's Hospital National Medical Center, George Washington University, Washington, DC (*S. Leikin,* CA-03888); Memorial Sloan-Kettering Cancer Center-Cornell University, New York, NY (*D. R. Miller,* CA-14557); Children's Hospital of Los Angeles, University of Southern California (*G. Higgins,* CA-02649); Babies Hospital, Columbia University, New York, NY (*J. Wolff,* CA-03526); Children's Hospital of Pittsburgh, University of Pittsburgh, Pa (*V. Albo,* CA-07439); Children's Hospital of Columbus, Ohio State University (*W. Newton,* CA-03750); Children's Orthopedic Hospital and Medical Center, University of Washington, Seattle (*R. Chard,* CA-10382); University of Wisconsin Hospitals, Madison (*N. Shahidi,* CA-05436); University of Minnesota Health Sciences Center, Minneapolis (*M. Nesbit,* CA-07306); Children's Memorial Hospital, Northwestern University, Chicago, Ill (*G. Honig,* CA-07431); University of Utah Medical Center, Salt Lake City (*E. Lahey,* CA-10198); Princess Margaret Hospital, University of Toronto, Canada (*M. Sonley,* Ontario Cancer Treatment and Research Foundation grant 41–17); Strong Memorial Hospital, University of Rochester, NY (*M. Klemperer,* CA-11174); Children's Hospital, University of Louisville, Ky (*D. R. Kmetz*); University of British Columbia, Vancouver, Canada (*M. Teasdale,* Vancouver Foundation); Children's Hospital of Philadelphia, University of Pennsylvania (*A. Evans,* CA-11796); James Whitcomb Riley Hospital for Children, Indiana University, Indianapolis (*R. Baehner,* CA-13809); New Jersey College of Medicine and Dentistry, Newark (*L. Vitale,* CA-12637); Harbor General Hospital, University of California, Los Angeles (*J. Finklestein,* CA-14560); University of California Medical Center, San Francisco (*A. Ablin,* CA-17829); Rainbow Babies and Children's Hospital, Case Western Reserve University, Cleveland, Ohio (*S. Gross,* CA-20320); Children's Hospital Medical Center, University of Cincinnati, Ohio (*B. Lampkin*); Vanderbilt University School of Medicine, Nashville, Tenn (*J. Lukens*); University of Oregon Health Sciences Center, Portland (*R. Neerhout*); University of Texas Health Science Center, San Antonio (*T. Williams*); University of California Medical Center, Los Angeles (*S. Feig*); and University of Iowa Hospitals and Clinics, Iowa City (*C. T. Kisker*). All CA grants are from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

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TABLE 1.—Diagnosis and response data*

Diagnosis	No. of patients treated	No. of patients with— Complete response/ partial response	No. of patients with— Progressive disease/ no response
Acute lymphocytic leukemia/acute undifferentiated leukemia	32	1	31
Acute myelogenous leukemia	15	0	15
Neuroblastoma	11	0	11
Ewing's sarcoma	2	0	2
Rhabdomyosarcoma	4	0	4
Miscellaneous	5	0	5

*Response criteria are noted in refs 8 and 9.

sis. In addition, cyclocytidine's antitumor activity is due not only to ara-C, but also to its direct inhibitory effect on L5178Y and L1210 leukemia cells in vitro (1).

Ara-C plasma levels are above therapeutic levels for > 6 hours when cyclocytidine is used in man. The plasma half-life of single doses of ara-C given by the iv, im, and sc routes is ≤ 1 hour (5).

In phase I and II trials, cyclocytidine has been reported to induce complete or partial remission in two of 25 adults with melanoma (6) and in two of five adults with myelogenous leukemia (7). Because cyclocytidine may be as effective as ara-C and may require less frequent intermittent administration, the Children's Cancer Study Group evaluated the agent in a phase II study between October 1975 and February 1978.

MATERIALS AND METHODS

Children (1–15 years of age) with histologic proof of malignancy and resistant to standard modalities of therapy, including ara-C, were eligible for inclusion in this study. Informed consent was obtained according to Department of Health, Education, and Welfare guidelines. Children with acute leukemia had to have > 25% blast cells in marrow aspirates.

Physical examinations and cbcs were required at least once weekly and bone marrow aspirations were performed prior to the initiation of therapy and at monthly intervals for children with acute leukemia. Bone marrow aspirations were optional prior to the initiation of therapy for patients with solid tumors. Evaluation of response to therapy was judged according to criteria previously published by the Children's Cancer Study Group (8,9).

Cyclocytidine was mixed with sterile water, normal saline, or 5% dextrose solution for injection, and was administered within 8 hours of reconstitution because no preservative was present. The requisite amount of drug was reconstituted with ≤ 5 ml of diluent and was administered in one or two sc injections. The area to be injected was often prepared with a cold compress to minimize any discomfort secondary to the injection. The drug was administered initially at a dose of 300 mg/m^2/day for 10 consecutive days. Since this dose appeared to be well-tolerated, it was increased to 450 mg/m^2/day and then to 600 mg/m^2/day for 10 days. All patients were to receive at least one course of therapy for 10 consecutive days. Whenever possible, second and subsequent courses were administered every 21 days for 10 consecutive days.

RESULTS

Seventy-three children were entered in the study. Four children were removed from the study prior to institution of drug treatment or had an inadequate trial defined as < 3 days of drug treatment. Six children (five with acute lymphocytic leukemia [ALL] and one with acute nonlymphocytic leukemia [ANLL]) had not received any ara-C prior to being entered in the study. All other patients with leukemia had received ara-C. None of the children with solid tumors entered in the study had received ara-C. The results are noted in table 1. The child who attained a complete remission had previously received intrathecal ara-C. The drug dose which resulted in this remission was 300 mg/m^2/day and was best documented on Day 54 of therapy; the remission duration was < 1 month.

The evaluation of administered drug doses is outlined in table 2. Eighteen of the 31 patients with ALL/acute undifferentiated leukemia who did not respond received two or more courses of the agent. One investigator administered a modified regimen of continuous therapy to two children with ALL (one child for 27 of 29 days and one child for 19 of 29 days). Although peripheral blood blast cells disappeared, neither child attained a remission. Patients with ANLL who did not respond included eight children who received two or more courses of therapy. In some courses, the drug was not administered according to protocol. This occurred for one of the fol-

TABLE 2.—Evaluation of administered drug doses

Diagnosis	Drug dose (mg/m^2/day)	No. of courses initiated/No. completed
Acute lymphocytic leukemia/acute undifferentiated leukemia	300*	47/38
	450	22/11
	600	12/10
Acute myelogenous leukemia	300	13/7
	450	5/4
	600	5/4
Solid tumors	300	18/15
	450	9/8
	600	11/7

*3 courses were given at > 200 mg/m^2/day, but < 300 mg/m^2/day.

lowing reasons: drug dose was increased or decreased midway in a course (nine courses), a course was stopped prior to completing 10 consecutive days (18 courses), the initial administered dose was < 300 mg/m^2/day (three courses), or the drug was continued for two or more courses without a rest period (two courses). Twenty-one of the 28 courses initiated at 600 mg/m^2/day were given for the complete 10-day period.

The major side effects were nausea and vomiting (ten patients), jaw pain (ten patients), thrombocytopenia (platelet count fell to 50,000/mm^3, four patients), granulocytopenia (absolute granulocyte count fell to < 500/mm^3, three patients), hypocellular marrow (three patients with leukemia), induration at the site of injection (two patients), generalized weakness (two patients), and one case each of dysphagia, mouth sores, generalized dermatitis, and hypotension with blurred vision. Nausea, vomiting, and jaw pain occurred at all three doses at about equal frequency. Leukopenia occurred only in children with leukemia or neuroblastoma in whom the marrow was infiltrated by tumor. Thrombocytopenia occurred in two children with solid tumors. Biochemical abnormalities were not documented. The side effects were managed with symptomatic therapy.

DISCUSSION

The preferred dosage of cyclocytidine has been the subject of controversy. Lokich et al (10) reported five trials in three children who received a 10-day course of cyclocytidine at a dose of 600 mg/m^2/day given iv and who developed moderate thrombocytopenia and leukopenia. In addition, there was one child who received cyclocytidine at a dose of 1000 mg/m^2 for 23 days. That child was granulocytopenic and thrombocytopenic throughout the therapy program. Burgess et al (6) found severe myelosuppression in adults at dose levels considerably < 600 mg/m^2/day. Our study suggests that a dose of 600 mg/m^2/day for 10 consecutive days is tolerated in children with various neoplastic disorders.

The most frequent side effects following cyclocytidine were nausea, vomiting, and jaw pain. Although hypotension has been reported as a major complication by others (7), there was only one case in our series.

Gehan and Schneiderman (11) have indicated that if 14 adequately treated patients with measurable disease fail to respond during a phase II trial of a new agent, there is less than a one-in-20 chance that the response rate for the drug will be > 20% in a larger and similar population. Since the dose and schedule used in this trial were the same as, or more aggressive than, those used in most other trials of this agent, it must be concluded that cyclocytidine is inactive against advanced ALL and ANLL in children previously exposed to ara-C. We noted successive failures in children with neuroblastoma and in smaller numbers of children with other tumors. The one response was in a patient with ALL who had received prior therapy with intrathecal ara-C.

Although cyclocytidine does have a prolonged elevation of plasma half-life, we were unable to demonstrate any superiority to ara-C. Since only five children with ALL and one with acute myelogenous leukemia had not received ara-C prior to being entered in the study, it is not possible to ascertain the comparative efficacy of cyclocytidine in ara-C-sensitive malignancies.

REFERENCES

1. Ho DHW, Rodriguez V, and Loo TL. Clinical pharmacology of O^2, 2′-cyclocytidine. Clin Pharmacol Ther 17:66–72, 1975.
2. Hoshi A, Kanazawa F, Kuretani K, et al. 2-2′*O*-Cyclocytidine, an antitumor cytidine analog resistant to cytidine deaminase. Gan 62:145–146, 1971.
3. Nakahara W, and Tokuzen R. Effect of 2,2′-*O*-cyclocytidine on transplanted lymphocytic sarcoma and reticulum cell sarcoma in mice. Gan 63:379–381, 1972.
4. Finklestein JZ, Arima E, Byfield PE, et al. Murine neuroblastoma: a model of human disease. Cancer Chemother Rep 57:405–412, 1973.
5. Finklestein JZ, Scher J, and Karon M. Pharmacologic studies of tritiated cytosine arabinoside (NSC-63878) in children. Cancer Chemother Rep 54:35–39, 1970.
6. Burgess MA, Bodey GP, Minow RA, et al. Phase I–II evaluation of cyclocytidine. Cancer Treat Rep 61:437–443, 1977.
7. Sakai Y, Konda C, Shimoyama M, et al. Cyclocytidine—a phase I study. Jpn J Clin Oncol 2:57–64, 1972.
8. Pierce M, Shore N, Sitarz A, et al. Cyclophosphamide therapy in acute leukemia of childhood. Cancer 19:1551–1560, 1966.
9. Finklestein JZ, Albo V, Ertel I, et al. 5-(3,3-Dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388) in the treatment of solid tumors in children. Cancer Chemother Rep 59:351–357, 1975.
10. Lokich JJ, Chawla PL, Jaffe N, et al. Phase I evaluation of cyclocytidine (NSC-145668). Cancer Chemother Rep 59:389–393, 1975.
11. Gehan E, and Schneiderman M. Experimental design of clinical trials. *In* Cancer Medicine (Holland J, and Frei E III, eds). Philadelphia, Pa, Lea and Febiger, 1973.

Stability and Preliminary Pharmacokinetic Studies of 1-(2-Chloroethyl)-3-[1-(5′-paranitrobenzoyl-2′,3′-isopropylidene)-α,β-D-ribofuranosyl]-1-nitrosourea (RFCNU), a Nonimmunosuppressive Nitrosourea [1,2]

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SUMMARY

Using high-performance liquid chromatography, the stability of RFCNU was monitored as a function of pH in aqueous buffers at 37°C and as a function of temperature in plasma. The kinetics of degradation of RFCNU are apparently first-order. The log *k*-pH profile demonstrated the hydroxyl ion-catalyzed solvolysis and a maximum stability around pH 3.0. This analytic assay was reliable for quantitating intact RFCNU in biologic fluids. After administration of 400 mg of RFCNU orally to a female patient, no intact drug was excreted in the urine and plasma levels were very low.

[Cancer Treat Rep 63:1335–1341, 1979]

Since the initial clinical and pharmacologic evaluation of BCNU by DeVita et al (1,2), a large variety of other chloroethylnitrosoureas have been tested in clinical trials as anticancer agents. Among these, the most widely tested are CCNU (3), methyl-CCNU (4), and chlorozotocin (5). More recently, RFCNU (fig 1) was synthesized (6). These drugs share several physicochemical and biochemical properties: they are highly lipid-soluble (except for the water-soluble chlorozotocin) and they decompose nonenzymatically when exposed to varying conditions (pH and temperature). Their biotransformation or degradation products bind to macromolecules through alkylation of nucleic acids and proteins (7,8) as well as through carbamoylation of proteins (9,10). However, despite their efficiency against metastases, they do possess undesirable side effects such as delayed bone marrow toxicity, characterized by aplasia and irreversible thrombocytopenia after six or seven therapy courses. All of them are immunosuppressive, except RFCNU (11), which has entered phase II clinical trials in France.

In order to optimize the dosage regimen, it is important to determine the pharmacokinetic parameters of RFCNU as well as its physicochemical properties. Thus, this paper will be confined to the stability studies of RFCNU in vitro, its specific dosage in biologic fluids by means of high-performance liquid chromatography (HPLC), and the monitoring of plasma levels and urinary excretion following oral administration to a female patient.

MATERIALS AND METHODS

RFCNU.—RFCNU was obtained from Prof. Imbach (Montpellier, France). After large-scale purification by means of HPLC (Waters Associates, Paris, France) on a Porasil column (Waters Associates) using chloroform as the eluent, its purity (> 95%) was checked on a 30-cm × 4-mm (internal diameter) μ-Bondapak C18 reversed-phase column (Waters Associates). The mobile phase was methanol:water (75:25; vol/vol) at a flow rate of 2 ml/minute. The detector wavelength was set at 254 nm. All separations were run isocratically. It is poorly soluble in methanol or ethanol (unstable) and is soluble in hexane,

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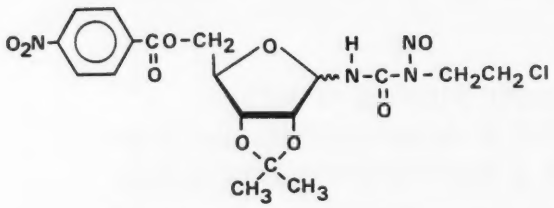

FIGURE 1.—Chemical structure of RFCNU.

ether, chlorinated solvents, and acetonitrile. Prior to use, the powder was dissolved in acetonitrile in which it is stable for days, even at room temperature.

Preparation of the internal standard.—In order to build calibration curves and avoid any variability due to the HPLC apparatus, we had to spike the samples with an internal standard. Four esters of paranitrobenzoic acid were prepared as follows: 500 mg of paranitrobenzoyl chloride (recrystallized from hexane), 10 ml of the alcohol to be esterified, and 0.5 ml of pyridine were heated under reflux for 3 hours. The excess alcohol was evaporated in vacuo and the residue purified on a column packed with 25 g of silica gel (Merck, Darmstadt, Germany) and eluted with chloroform. Isopropanol, isoamyl alcohol, *n*-heptanol, and *n*-octanol were used and gave the pure corresponding esters, as monitored by HPLC (fig 2). The *n*-heptyl ester (E-4) was used in all our experiments since it did not interfere with any of the endogenous plasma peaks or with RFCNU degradation products (fig 3).

RFCNU extraction from pure water and aqueous buffers.—Five-ml aliquots of water or 5×10^{-3} M buffers (1 liter of water or buffer was spiked with 1 mg of

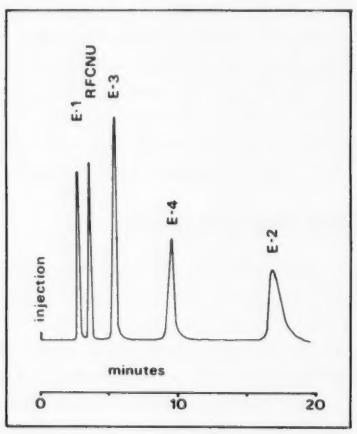

FIGURE 2.—High-performance liquid chromatogram of RFCNU and of the different paranitrobenzoic esters. E-1 = isopropyl; E-2 = isoamyl; E-3 = *n*-octyl; E-4 = *n*-heptyl. The ultraviolet absorption is measured as a function of time.

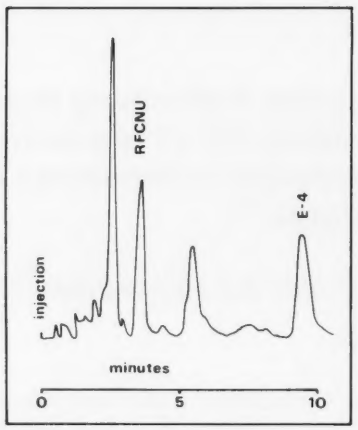

FIGURE 3.—High-performance liquid chromatogram of RFCNU, its degradation products, and the internal standard, *n*-heptyl paranitrobenzoate (E-4).

RFCNU dissolved in 2 ml of acetonitrile) at different pH values (table 1) are poured over 150 µl of 0.5 M citric acid in 15-ml centrifuge tubes. The mixture is then extracted with 2 × 2 ml of dichloromethane. The organic phase is evaporated to dryness under a nitrogen stream and the residue taken up with 120 µl of an internal standard solution in acetonitrile. One hundred µl is then injected into the column and peak height ratios are calculated. From water, the calibration curve thus obtained is a straight line over a wide concentration range (from 15 µg/ml to 10 ng/ml). The intercept is not significantly > 0 and the reproducibility of the assay method from day to day is good and quite sensitive; the detection limit of pure material is 10^{-9} g (about 2 pmols) injected directly into the chromatograph.

RFCNU extraction from plasma.—To 1.0 ml of spiked fresh human plasma samples, 2 ml of cold (0°C–4°C)

TABLE 1.—Degradation rate constants (k) and chemical half-lives of RFCNU as a function of pH at 37°C in 5×10^{-3} M buffers

pH (buffer)	k (mins^{-1})	Half-life (mins)
1.2 (HCl)	3.13×10^{-2}	22
2.9 (citrate)	4.83×10^{-4}	1435
4.0 (citrate)	7.30×10^{-4}	950
5.0 (citrate)	3.73×10^{-3}	186
5.1 (Tris-HCl)	4.60×10^{-3}	151
6.0 (phosphate)	2.96×10^{-2}	23
6.7 (phosphate)	0.117	5.9
7.2 (phosphate)	0.262	2.6
7.2* (phosphate)	0.253	2.7
7.4 (phosphate)	0.360	1.9

*0.15 M NaCl.

hexane containing 1% isoamyl alcohol is added. The tube is shaken for 30 seconds on a Vortex mixer and then centrifuged (4°C) at 3000 rpm for 2 minutes. The organic phase is collected (1.8 ml). The extraction step is repeated, the organic extracts are combined and evaporated under a stream of nitrogen at room temperature (22°C), and the residue is reconstituted in 120 µl of an internal standard solution in methanol:water (75:25; vol/vol). One hundred µl is then injected into the column and peak height ratios are calculated. The calibration curve is a straight line over the 10–100-ng/ml concentration range with no intercept and a standard error of < 5% for triplicates. The lower sensitivity is 5 ng/ml with a 10% standard error.

RFCNU extraction from urine.—A 0.5 M citric acid solution (0.5 ml) is added to 5-ml aliquots of spiked urine samples to get a pH of between 3.0 and 4.0. The extraction is carried out with 8 ml of hexane containing 1% isoamyl alcohol by shaking for 1 minute and centrifugation for 5 minutes at 3000 rpm. Five ml of the organic phase is collected and evaporated to dryness, and the residue is reconstituted in 120 µl of internal standard solution and chromatographed. The calibration curve is a straight line over the 5–30-ng/ml concentration range.

Degradation kinetic studies.—To a 1.0-liter buffer solution kept in subdued light at 37°C (water bath), 1 mg of RFCNU in 2 ml of acetonitrile is added. At different times, 5-ml aliquots are collected and extracted as described above and unchanged RFCNU is quantitated. The results are expressed as the percentage of intact drug against time (figs 4–7). Additional kinetic studies were done in plasma at different temperatures (figs 8 and 9).

Pharmacokinetic study.—A 38-year-old female patient (38 kg; 1.25 m^2 body surface area) with an adenocarcinoma and metastases of pancreatic or digestive origin was given four gelatin capsules orally, containing 100 mg of RFCNU and 45 mg of lactose each, with a glass of water. Seventeen 4-ml blood samples were withdrawn over 4 hours (blank, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, and 240 minutes) by means of a butterfly needle placed in a cubital vein. The catheter was heparinized and thus had to be rinsed with 0.5–1.0 ml of blood prior to sampling in order to avoid any dilution. Blood was collected in 5-ml tubes containing lithium heparinate. The tubes were plunged into ice water immediately after collection and centrifuged at 4°C for 10 minutes at 3000 rpm. Plasma was then extracted as described above. Urine was collected by means of a urinary catheter. The volume of each collection was measured and 5-ml aliquots were treated as described. The HPLC analysis of the contents of a capsule of the same batch has shown that RFCNU was intact.

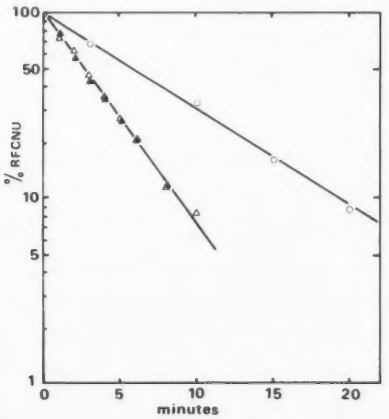

FIGURE 4.—Kinetics of degradation of RFCNU in 5×10^{-3} M phosphate buffers at 37°C. ○ = pH 6.7; ▲ = pH 7.2; △ = pH 7.2 plus 0.15 M NaCl.

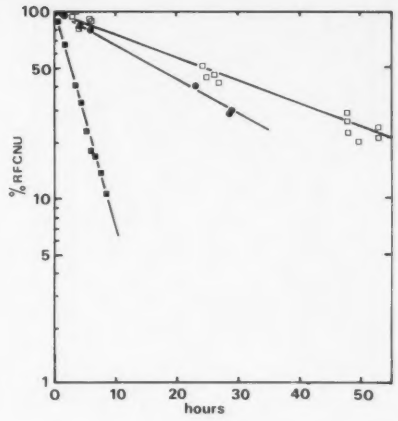

FIGURE 5.—Kinetics of degradation of RFCNU at 37°C. □ = citrate buffer, pH 2.9; ◒ = citrate buffer, pH 4.0; ■ = Tris-HCl buffer, pH 5.1.

RESULTS

Kinetic studies.—Semilogarithmic plots (figs 4–7) of the percentage of intact RFCNU as a function of time at different pH values give straight lines, thus indicating that the degradation reaction under these conditions is apparently first-order. The calculated slopes of these curves by means of logarithmic regression are the apparent first-order rate constants (k) expressed in minutes $^{-1}$. The corresponding chemical half-life is given by the expression:

$$\text{half-life} = \frac{\ln 2}{k} = \frac{0.693}{k}.$$

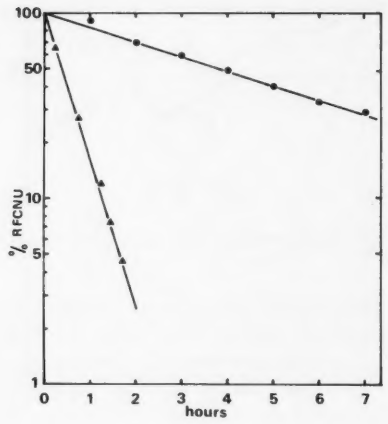

FIGURE 6.—Kinetics of degradation of RFCNU at 37°C. ▲ = phosphate buffer, pH 6.0; ● = citrate buffer, pH 5.0.

The results are summarized in table 1. The log k-pH profile for the solvolysis of RFCNU is given in figure 10, based on the data shown in table 1. The influence of temperature upon the degradation rate in pooled human plasma was also studied. At 37°C, the chemical half-life is 3.5 minutes, increasing to 25 minutes at 24°C and to 18 hours at 0°C (table 2). The logarithmic version of the Arrhenius expression is:

$$\log k = -\frac{\Delta H a}{2.303\ RT} + \log P.$$

The Arrhenius plot for the data of the solvolysis of RFCNU is given in the insert to figure 9, based on the data shown in table 2. The pertinent ΔHa, the energy of activation of the reaction, and the log P, related to the

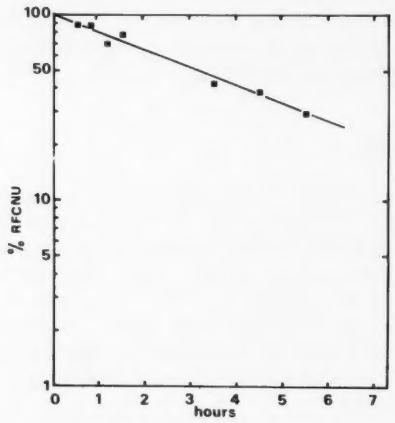

FIGURE 7.—Kinetics of degradation of RFCNU in HCl (pH 1.2) at 37°C.

TABLE 2.—Chemical half-lives of RFCNU in plasma as a function of temperature

Temperature (°C)	1/T (K^{-1})	Half-life (secs)	ln k
0	3.66×10^{-3}	66×10^{3}	− 11.46
24	3.37×10^{-3}	1500	− 7.68
37	3.23×10^{-3}	210	− 5.71

entropy of the system, are 25.4 kcal mol^{-1} and 15.4 respectively.

Pharmacokinetic study.—After collection of blood samples, rapid analysis of unchanged RFCNU in plasma by means of HPLC gave the following results: 4.2, 8.0, 13.0, and 3.6 ng/ml at 20, 25, 30, and 40 minutes respectively. No RFCNU was recovered in the urine in its intact form. Further in vitro experiments in whole blood and with rbcs have shown that there was no significant distribution into the erythrocytes.

DISCUSSION

A survey of the literature indicates that most of the physiologic disposition studies of the chloroethylnitro-

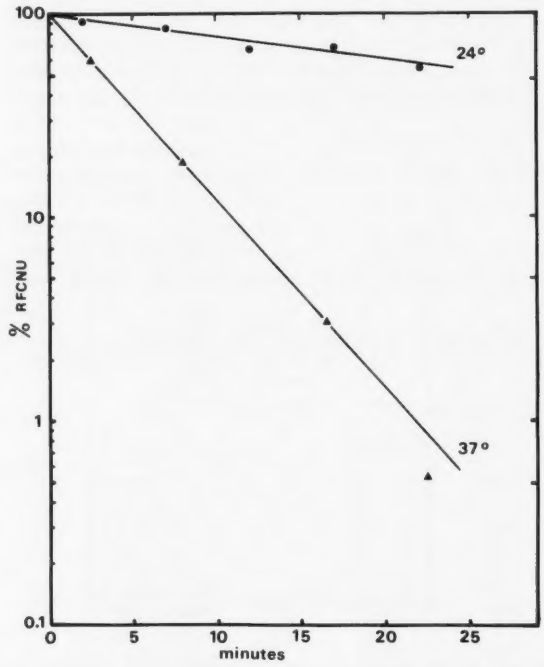

FIGURE 8.—Kinetics of degradation of RFCNU in plasma.

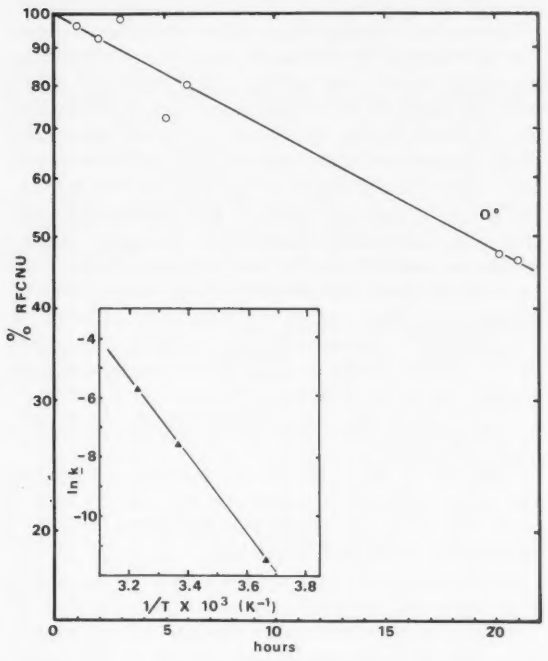

FIGURE 9.—Kinetics of degradation of RFCNU in plasma at 0°C; insert shows the Arrhenius plot.

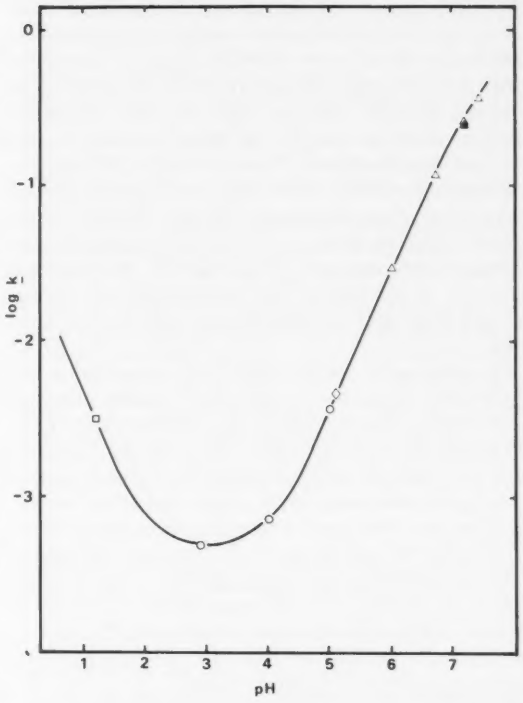

FIGURE 10.—Log *k*-pH profile for RFCNU in different buffers. □ = HCl; ○ = citrate; ◇ = Tris-HCl; Δ = phosphate; ▲ = phosphate plus 0.15 M NaCl.

soureas in animals and man were done using radiolabeled compounds. In one study the authors did not separate the different species in biologic samples (12), and therefore those studies did not give much insight into the blood and tissue levels of the intact drug (chlorozotocin). Others have used thin-layer chromatography to separate the drug, ie, BCNU (2), CCNU and methyl-CCNU (13), and chlorozotocin (14), from its degradation products. A more recent study (15) describes the quantitative analysis of BCNU by means of chemical ionization-mass spectroscopy. Since, in France, we are not allowed to use radiolabeled drugs in man, we had to develop a cold-sensitive and -specific assay for RFCNU.

The paranitrobenzoyl ester has a maximal absorption wavelength, λmax, of 255 nm (ε = 18,000), and the resulting mixture after treatment with alkali had a λmax of 258 nm. From our preliminary stability studies, blood levels were expected to be very low and, in that case, the Bratton-Marshall method (16) would not be sensitive enough (maximum sensitivity, 10^{-5} M). Also, the paranitrobenzyl pyridine test (17) is not sensitive enough for our study. Furthermore, it can only be used for the estimation of the in vitro alkylating activity since it is not specific for the intact drug and also reacts with other alkylating moieties such as chloroethanol that might be present in plasma. The use of HPLC proved to be very convenient in our case, since intact RFCNU could be separated from its degradation or biotransformation products and quantitated by ultraviolet detection.

We have now undertaken additional studies in order to identify the major degradation products both in vitro and in vivo. In vitro studies have shown that chloroethanol is a derivative of the chloroethyl moiety as monitored by gas chromatography. However, the chemical structures of the compounds detected by HPLC (fig 3) are not yet fully identified.

From the semilogarithmic plots, we get the log *k*-pH profile for the solvolysis of RFCNU. At pH 7.4, the chemical half-life is < 2 minutes (phosphate buffer). Between pH 4.5 and 7.0, specific hydroxyl ion-catalyzed solvolysis is indicated by the fact that the slope of the log *k*-pH plot in that region approaches a slope of +1. At highly acidic values (pH < 1.5), decomposition also occurs but the mechanism is different: the protective groups (isopropylidene and paranitrobenzoate) are probably hydrolyzed. It has also been reported (18) that in the case of BCNU, CCNU, and streptozotocin, the nitroso group is cleaved at acidic pHs. Around physiologic pH (7.2), we did not find any significant influence of the ionic strength (table 1 and fig 4). In plasma, the chemical half-life of

RFCNU is longer (3.5 minutes) than in buffered water (1.9 minutes) but is much shorter than that of BCNU in blood serum (45 minutes) or that of CCNU in phosphate buffer at pH 7.2 (64 minutes) (19). More recently, Levin et al (20) reported different values for BCNU chemical half-lives at pH 7.4 and 37°C in different media, ie, 11.6 ± 0.5 minutes in normal volunteer sera and 16.7 minutes in normal sera 3 hours after a fatty meal, indicating that lipid content of the plasma may decrease the rate of degradation of nitrosoureas in serum. Studies are underway to prepare liposome-entrapped RFCNU[6]; our assay will be useful in comparing the bioavailability of the unchanged drug after administration of different formulations.

The influence of temperature on the degradation rate of RFCNU in plasma was checked in order to handle blood samples collected during the pharmacokinetic study. The energy of activation (ΔHa = 25.4 kcal mol^{-1}) and the log P (15.4) are in good agreement with those reported for other nitrosoureas (18,21). Based on these results, blood samples were worked up at low temperatures (0°C–4°C) during the processing of the samples. Analysis of spiked blood samples following this procedure has shown that the yield of recovery of intact RFCNU was 53% ± 4% (n = 4) at concentrations around 50 ng/ml. The organic extracts were stable at –20°C for at least 1 day and every sample was analyzed within 24 hours.

Since the solubility of RFCNU in water or in saline is very low, approximately 4 mg/ml, the drug cannot be administered iv. In man, it is given orally and has proved to be active in digestive tract tumors (22). As expected, blood levels were very low since the drug is rapidly degraded in the blood (chemical half-life, 3.5 minutes). Furthermore, when it crosses the liver, RFCNU may undergo an important first-pass effect. From the studies of May et al (23) and Hill et al (24) on the biotransformation of nitrosoureas, we know that these drugs are highly metabolized in the liver. Both processes, degradation and metabolism, account for the fact that, using a specific assay (2,15,25), no intact BCNU was found in plasma when the drug was given orally.

In our case, the hydrolysis of the isopropylidene group and/or the paranitrobenzoyl ester might occur in gastric juice, liberating the unsubstituted ribosyl chloroethylnitrosourea. This compound was found to be far less stable than RFCNU itself when first synthesized (6) as an analog of chlorozotocin. We must then postulate that RFCNU, like other nitrosoureas, acts as a prodrug liberating the active alkylating species. Preliminary autoradiographic studies[7] have shown that only the chloroethyl moiety distributes into the cerebrospinal fluid and the brain as does chlorozotocin (12). The rate of release of the active part of the nitrosoureas is probably influenced by a number of physicochemical and biochemical parameters, eg, solubility, structure of the carbamoylating moiety, and affinity for cytochrome P-450. More detailed studies on the circulating active species are underway in our laboratory to better understand their mechanism of action and/or toxicity. It is significant that, after administration of chlorozotocin to animals, 40% of the radioactivity corresponding to the chloroethyl chain was found in bile, probably as conjugates of chloroethanol which are later hydrolyzed in the intestine and then re-absorbed (enterohepatic cycle), since almost no radioactivity was found in the feces (12). The study of the fate of the chloroethyl moiety seems to be the key to the problem. This is supported by the results of Reed and May (26) who isolated dithioacetic acid and *S*-carboxymethylcysteine from urine of rats after administration of CCNU.

Oxidation of chloroethanol would produce chloroacetaldehyde, which is well-known as a potent alkylator of thiols (cysteine, for example); subsequent oxidation would give the carboxyl function.

Furthermore, in many systems, cytotoxicity appears to be determined primarily by the alkylating activity of chloroethylnitrosoureas rather than by their carbamoylating activity. The exact biologic significance of the carbamoylation of macromolecules is still debated (27–32).

The most important feature, however, is that the concentration of intact nitrosoureas found in blood and tissues is less than that needed to kill cells in culture: RFCNU concentrations of 5×10^{-5} M to 5×10^{-4} M are needed against HeLa-S3 cells (33), BCNU, CCNU, or chloroethylnitrosourea concentrations of 10^{-5} M are needed against L1210 cells (34), and BCNU concentrations of 5×10^{-5} M are needed against WI-38 and VA-13 cells (35). Therefore, in the body, the intact drug may never reach its intended site of action but its pharmacologically active degradation products may circulate in the bloodstream under a state that remains to be determined. We feel that more detailed metabolic studies and pharmacokinetic experiments with the major metabolites identified as to their distribution in tissues (tumor and bone marrow) will give us guidelines for the design of new compounds in these series and appropriate treatment schedules. Until now, structure-activity relationships (36–39) have not proved very successful in developing new chloroethylnitrosoureas, except that the presence of a sugar radical seems to improve the therapeutic index. Chlorozotocin and RFCNU are both active in man, but differ in their physicochemical properties; chlorozotocin is water-soluble and RFCNU is water-insoluble.

REFERENCES

1. DeVita VT, Carbone PP, Owens AH, Jr, et al. Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea, NSC-409962. Cancer Res 25:1876–1881, 1965.

[6]Lussan C. Personal communication, 1978.

[7]Madelmont JC. Personal communication, 1978.

2. DeVita VT, Denham C, Davidson JD, et al. The physiological disposition of the carcinostatic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in man and animals. Clin Pharmacol Ther 8:566–577, 1967.
3. Wasserman TH, Slavik M, and Carter SK. Review of CCNU in clinical cancer therapy. Cancer Treat Rev 1:131–151, 1974.
4. Wasserman TH, Slavik M, and Carter SK. Methyl-CCNU in clinical cancer therapy. Cancer Treat Rev 1:251–269, 1974.
5. Schein PS, Panasci L, Woolley PV, et al. Pharmacology of chlorozotocin (NSC-178248), a new nitrosourea antitumor agent. Cancer Treat Rep 60:801–805, 1976.
6. Montero JL, and Imbach JL. Synthèse de nouvelles nitrosourées à visées antinéoplasiques. C R Acad Sci 279:809–811, 1974.
7. Kohn KW. Interstrand cross-linking of DNA by 1,3-bis(2-chloroethyl)-1-nitrosourea and other 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 37:1450–1454, 1977.
8. Thomas CB, Osieka R, and Kohn KW. DNA cross-linking by in vivo treatment with 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea of sensitive and resistant human colon carcinoma xenografts in nude mice. Cancer Res 38:2448–2454, 1978.
9. Connors TA, and Hare JR. The binding of ^{14}C-labelled 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) to macromolecules of sensitive and resistant tumors. Br J Cancer 30:477–480, 1974.
10. Kahn HE, Jr, Kohn KW, and Lyles JM. Inhibition of DNA repair by the 1,3-bis(2-chloroethyl)-1-nitrosourea breakdown product, 2-chloroethyl isocyanate. Cancer Res 34:398–402, 1974.
11. Imbach JL, Montero JL, Moruzzi A, et al. The oncostatic and immunosuppressive action of new nitrosourea derivatives containing sugar radicals. Biomedicine 23:410–413, 1975.
12. Plowman J, Dingell JV, and Adamson RH. Disposition of chlorozotocin in rats and dogs. Cancer Treat Rep 62:31–44, 1978.
13. Sponzo RW, DeVita VT, and Oliverio VT. Physiologic disposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU) in man. Cancer 31:1154–1159, 1973.
14. Mhatre RM, Green D, Panasci LC, et al. Pharmacologic disposition of chlorozotocin in mice. Cancer Treat Rep 62:1145–1151, 1978.
15. Weinkam RJ, Wen JHC, Furst DE, et al. Analysis for 1,3-bis(2-chloroethyl)-1-nitrosourea by chemical ionization mass spectrometry. Clin Chem 24:45–49, 1978.
16. Loo TL, and Dion RL. Colorimetric method for the determination of 1,3-bis(2-chloroethyl)-1-nitrosourea. J Pharm Sci 54:809–810, 1965.
17. Wheeler GP, and Chumley S. Alkylating activity of 1,3-bis(2-chloroethyl)-1-nitrosourea and related compounds. J Med Chem 10:259–261, 1967.
18. Garrett ER. Prediction of stability in pharmaceutical preparations. VII. The solution degradation of the antibiotic streptozotocin. J Pharm Sci 49:767–777, 1960.
19. Montgomery JA, James R, McCaleb GS, et al. The modes of decomposition of 1,3-bis(2-chloroethyl)-1-nitrosourea and related compounds. J Med Chem 10:668–674, 1967.
20. Levin VA, Hoffman W, and Weinkam RJ. Pharmacokinetics of BCNU in man: a preliminary study of 20 patients. Cancer Treat Rep 62:1305–1312, 1978.
21. Garrett ER, Goto S, and Stubbins JF. Kinetics of solvolysis of various *N*-alkyl-*N*-nitrosoureas in neutral and alkaline solutions. J Pharm Sci 54:119–123, 1965.
22. Mathé G, Serrou B, Hayat M, et al. Preliminary results of phase I and II clinical trials of RFCNU, a new nitrosourea sugar derivative, in digestive tract tumors. Biomedicine 27:294–297, 1977.
23. May HE, Boose R, and Reed DJ. Hydroxylation of the carcinostatic 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) by rat liver microsomes. Biochem Biophys Res Commun 57:426–433, 1974.
24. Hill DL, Kirk MC, and Struck RF. Microsomal metabolism of nitrosoureas. Cancer Res 35:296–301, 1975.
25. Oliverio VT. Pharmacology of the nitrosoureas: an overview. Cancer Treat Rep 60:703–707, 1976.
26. Reed DJ, and May HE. Alkylation and carbamoylation intermediates from the carcinostatic 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU). Life Sci 16:1263–1270, 1975.
27. Fox PA, Panasci LC, and Schein PS. Biological and biochemical properties of 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (NSC D 254157), a nitrosourea with reduced bone marrow toxicity. Cancer Res 37:783–787, 1977.
28. Fornace AJ, Jr, Kohn KW, and Kahn HE, Jr. Inhibition of the ligase step of excision repair by 2-chloroethyl isocyanate, a decomposition product of 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 38:1064–1069, 1978.
29. Heal J, Fox P, Sinks LF, et al. Effect of carbamoylation on repair of nitrosourea (NU) alkylated DNA in L1210 cells. Proc Am Assoc Cancer Res and ASCO 19:202, 1978.
30. Babson JR, Reed DJ, and Sinkey MA. Active site specific inactivation of chymotrypsin by cyclohexyl isocyanate formed during degradation of the carcinostatic 1-(2-chlorohexyl)1-nitrosourea. Biochemistry 16:1584–1589, 1977.
31. Babson JR, and Reed DJ. Inactivation of glutathione reductase by 2-chloroethyl nitrosourea-derived isocyanates. Biochem Biophys Res Commun 83:754–762, 1978.
32. Gross M, Whetzel NK, and Folk JE. Alkylisocyanates as active-site-directed inactivators of guinea pig liver transglutaminase. J Biol Chem 250:7693–7699, 1975.
33. Moruzzi A. Synthèse et activité biologique d'une série de nouvelles drogues à visée oncostatique: les glycosyl-nitrosourées. Thesis, Montpellier, France, 1976.
34. Kahn HE, Jr. Comparison of biochemical and biological effects of four nitrosoureas with different carbamoylating activities. Cancer Res 38:2363–2366, 1978.
35. Erickson LC, Bradley MO, and Kohn KW. Strand breaks in DNA from normal and transformed human cells treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. Cancer Res 37:3744–3750, 1977.
36. Wheeler GP, Bowdon BJ, Grimsley JA, et al. Interrelationships of some chemical, physicochemical, and biological activities of several 1-(2-haloethyl)-1-nitrosoureas. Cancer Res 34:194–200, 1974.
37. Panasci LC, Green D, Nagourney R, et al. Structure-activity analysis of chemical and biochemical parameters of chloroethylnitrosoureas in mice. Cancer Res 37:2615–2618, 1977.
38. Montgomery JA. Chemistry and structure-activity studies of the nitrosoureas. Cancer Treat Rep 60:651–664, 1976.
39. Schein PS, Heal J, Green D, et al. Pharmacology of nitrosourea antitumor agents. *In* Fundamentals in Cancer Chemotherapy, Antibiotics and Chemotherapy (Schabel FM, Jr, ed). Basel, Switzerland, Karger, 1978, vol 23, pp 64–75.

Phase II Evaluation of Vindesine in Patients With Non-Small Cell Carcinoma of the Lung [1,2,3]

Richard J. Gralla,* Bruce G. Raphael, Robert B. Golbey, and Charles W. Young [4,5]

SUMMARY

Fifty-two patients with non-small cell carcinoma of the lung were treated iv with doses of vindesine at 3–4 mg/m²/week iv. Partial responses occurred in all histologic types in ten of 46 adequately treated patients, for an overall response rate of 22%. Patients not previously treated with chemotherapy had a higher response rate than those who had received prior chemotherapy (33% versus 12%). Reversible peripheral neuropathy occurred in all patients, and was generally of a mild to moderate degree. Mild leukopenia was seen frequently, with a median wbc nadir of 2900/mm³. As reported with the older vinca alkaloids, platelet-sparing with occasional episodes of thrombocytosis occurred with vindesine. It is concluded that vindesine is an active agent in non-small cell carcinoma of the lung and that further studies in previously untreated patients are indicated.

[Cancer Treat Rep 63:1343–1346, 1979]

Conventional chemotherapy for non-small cell lung carcinoma (NSCLC) has remained unsatisfactory. Protocols utilizing four- and five-drug combinations, with or without radiotherapy, have produced objective remissions. These responses, however, are generally of short duration, have only a minor effect on the median survival of the treatment groups, are accompanied by moderate to marked toxicity, and appear to have little advantage in subsequent larger studies when randomized against less toxic regimens (1–6). For these reasons, emphasis at the Memorial Sloan-Kettering Cancer Center has been on the testing of new agents in NSCLC.

Observations of objective remissions in NSCLC with vindesine in early clinical trials (7,8) encouraged the further study of this agent despite the marginal activity of the older vinca alkaloids seen in studies conducted in the 1960s (9–12). Vindesine is a semi-synthetic analog of vinblastine and is similar to this agent in its distribution and metabolism (7). However, the activity of vindesine in murine hematologic and solid tumor models more closely resembles that of vincristine (7,13,14). In addition, in vitro studies of axoplasmic transport in the sciatic nerve have indicated that vindesine has less neurotoxicity than vincristine (15).

Phase I studies with vindesine determined that doses of 3–4 mg/m² given weekly by rapid iv injection produced manageable toxicity; leukopenia and peripheral neuropathy were the most commonly observed dose-limiting effects (7,16,17). Recent phase II trials have reported objective responses in patients with breast carcinoma (18), melanoma (18), esophageal carcinoma (19), testicular carcinoma (20), acute lymphocytic leukemia, chronic myelocytic leukemia, and Hodgkin's and non-Hodgkin's lymphomas (21). This paper reports the results of a phase II trial of vindesine in patients with NSCLC.

MATERIALS AND METHODS

Between February 1977 and March 1978, 52 patients were entered in this study. All patients had objectively measurable disease and histologic confirmation of the diagnosis of NSCLC. A performance status (Karnofsky

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TABLE 1.—Characteristics and responses in 46 patients treated with vindesine

Characteristic	All patients	Patients with PR	Patients with MR
Median performance status (range)	60(50–90)	70(50–80)	60(50–80)
Median age in yrs (range)	59(32–78)	63(56–75)	50(32–72)
Males:females	31:15	5:5	4:3
Prior treatment status			
No prior treatment	10	3	2
Radiation therapy only	11	4	1
Chemotherapy only	15	3	3
Chemotherapy and radiation therapy	10	0	1
Histologic type			
Adenocarcinoma	29	6	5
Epidermoid carcinoma	14	3	1
Large cell undifferentiated carcinoma	3	1	1
Totals	46	10	7

scale) of ≥ 50%, a platelet count of ≥ 120,000/mm^3, and a wbc count of ≥ 4000/mm^3 were required for entry in this trial. Patient characteristics are shown in table 1. None of the 25 patients who had received prior chemotherapy had been previously treated with vinca alkaloids. Informed consent was obtained from all patients.

Before starting vindesine (supplied by Eli Lilly and Co, Indianapolis, Ind), all patients had a complete history and physical examination and a laboratory evaluation including: cbc, 12-channel biochemical screening profile, 5′-nucleotidase, serum electrolytes and creatinine, prothrombin time, and chest roentgenogram. Bone and liver scans and computerized transaxial tomography were performed only when clinically indicated. All patients had weekly physical examinations and cbcs, biweekly determinations of biochemical tests, and monthly evaluations of chest roentgenograms unless indicated at an earlier date.

Adequate treatment required a minimum of three weekly doses of vindesine as well as dose-limiting toxicity in terms of leukopenia or neurotoxicity. Response criteria were defined as: *complete remission*—disappearance of all evidence of disease for ≥ 1 month; *partial remission (PR)*—> 50% decrease of all measurable lesions for ≥ 1 month; *minor response (MR)*—objective tumor decrease of > 25%, but less than that required for a PR; and *progression*—objective increase in measurable disease, or the appearance of new tumor lesions.

Vindesine was administered as a rapid injection through an established free-flowing iv line. The initial dose was 3.0 mg/m^2, which was increased by 0.5 mg/m^2 each week (but not exceeding 4.0 mg/m^2/week, total dose) in the absence of myelosuppression. The dose was attenuated by 50% if the wbc count was 2000–2999/mm^3 or if the platelet count was 75,000–100,000/mm^3; chemotherapy was withheld for 1 week if the blood counts were lower than these levels. After ten weekly injections, responding patients were placed on a maintenance schedule of 4 mg/m^2 every 2 weeks.

RESULTS

Therapeutic Responses

PRs occurred in ten of the 46 adequately treated patients, for an overall response rate of 22%. Seven additional patients experienced MRs. Complete remissions were not observed in this study. In all responding patients, objective evidence of improvement occurred within the first month of treatment. As shown in table 1, remissions were noted in all World Health Organization non-small cell histologic categories and occurred with equal frequency (PR rate, 21%) in epidermoid and adenocarcinoma. Seven of the ten patients with PRs had more than one site of measurable tumor. Responses were observed in the lung (nine patients), lymph nodes (three patients), liver (two patients), pleura (two patients), and bone (one patient).

Extensive prior treatment, especially with chemotherapy, appeared to have a negative influence on the PR rate. Only three (12%) of 25 patients who had received prior chemotherapy had a PR. A PR rate of 33% was observed for the 21 patients who had not previously received chemotherapy. Prior treatment with radiotherapy only did not appear to affect the response rate.

Duration of PRs ranged from 2 to 9 months, with a median of 5 months. The ten patients with PRs had a median survival of 9 months, while the nonresponding patients and those with MRs had a median survival of 4 months.

TABLE 2.—Toxicity in 46 patients treated with vindesine

Toxic effect	No. of patients (%)
Constipation	40(87)
Alopecia	28(61)
Fever	24(52)
Nausea or vomiting	6(13)
Superficial phlebitis	6(13)
Wbc count nadir (cells/mm^3)	
≥ 4000	10(22)
3000–3999	12(26)
2000–2999	16(35)
1000–1999	7(15)
<1000	1(2)
Platelet count nadir (cells/mm^3)	
≥150,000	39(85)
125,000–149,999	4(9)
100,000–124,999	2(4)
<100,000	1(2)

Toxicity

The median number of doses given was six per patient (range, three to 33 doses). Leukopenia was the most common manifestation of myelosuppression (table 2) in this study. Although the leukopenia was frequently dose-limiting, < 20% of patients experienced wbc counts of < 2000/mm^3 (median nadir, 2900/mm^3; range, 500–6800/mm^3). Thrombocytopenia was an unusual occurrence; < 10% of patients had platelet counts of < 125,000/mm^3 (median nadir, 260,000/mm^3; range, 91,000–488,000/mm^3). As previously observed with older vinca alkaloids (22), platelet-sparing and occasional instances of thrombocytosis were noted in this study. A mean 20% increase in platelet counts and a concurrent mean 35% decrease in wbc counts occurred in those patients who received full doses of vindesine.

Peripheral neuropathy was the most prominent toxic effect in this trial. Table 3 shows the observed neurotoxicity which occurred in all patients within three to four doses. Patients with marked (3+) toxicity received a median of 30% more vindesine than patients with moderate (2+) symptoms, and received a median of 140% more drug than those with mild (1+) complaints. Thus, the degree of neurotoxicity generally appeared to be dose-related. The neuropathy was reversible, but required several months without vindesine for the clearing of all symptoms. Patients receiving the every-second-week maintenance schedule appeared to have no further progression of their neuropathy.

Table 2 lists other toxic reactions observed in this study. Constipation, occurring in 87% of patients, was usually mild. Alopecia (> 25% hair loss) was common, and fever in the range of 38°C–39°C occurred within 48 hours of treatment in the majority of patients. There were no drug-related deaths.

DISCUSSION

In view of the continued difficulty in the treatment of advanced NSCLC, the observed major response rate of 22% in this phase II study with vindesine appears encouraging. Of particular note is the response rate of 33% in the 21 patients previously untreated with chemotherapy. This advantage for untreated patients correlates well with the observations of Smith et al (18), who noted a major response rate of 29% with vindesine in breast cancer, with five of the six responses occurring in patients who had not received prior chemotherapy.

While the current study demonstrates objective responses with vindesine, it does not address the question of whether or not vindesine has a superior response rate in NSCLC compared with the older vinca alkaloids. A review of vinblastine phase II studies in this disorder reported a major response rate of 11% (23), with response

TABLE 3.—Neurotoxicity in 46 patients treated with vindesine

Grade	Description		Median total dose (mg/m^2)	No. of patients	(%)
4+	Paraplegia; prolonged paralytic ileus		—	0	
3+	Muscle weakness; decrease in manual dexterity	Interfering with normal functioning	45.5	5	(11)
2+	Muscle weakness; prolonged dysesthesias	Not interfering with normal functioning	35	11	(24)
1+	Decrease in deep tendon reflexes; paresthesias; jaw pain; myalgias		19	30	(65)
0+	No toxicity		—	0	

rates in separate trials ranging from 0 (9) to 20% (10). Few studies with vincristine as a single agent in lung cancer have been performed. A response rate of 5% in 37 patients was found using vincristine in a cooperative trial (12), and a more recent study in previously untreated patients reported a response rate of 17% (24).

The toxicity experienced with vindesine has been easily managed. Neurotoxicity appears to be cumulative on the weekly schedule, but generally does not progress in patients maintained on every-other-week dosages; it is reversible after discontinuation of treatment. This toxicity is similar to that observed with vincristine, but may be somewhat less than that observed with the older agent. Vindesine induced mild to moderate leukopenia; only one patient experienced a wbc count of $< 1000/mm^3$. The platelet-sparing effect could have potential value in the use of vindesine with other chemotherapeutic agents.

In addition to an objective response rate similar to that observed in several studies using combinations of conventional agents in NSCLC (2–6), vindesine has the advantages of rapid onset of response and relatively mild toxicity. We conclude that further trials using vindesine, alone and in combination, in previously untreated patients with NSCLC are indicated.

REFERENCES

1. Livingston RB. Combination chemotherapy of bronchogenic carcinoma. I. Non-oat cell. Cancer Treat Rev 4:153–165, 1977.
2. Bodey GP, Lagakos SW, Gutierrez AC, et al. Therapy of advanced squamous carcinoma of the lung. Cancer 39:1026–1031, 1977.
3. Livingston RB, Heilbrun L, Lehane D, et al. Comparative trial of combination chemotherapy in extensive squamous carcinoma of the lung: a Southwest Oncology Group study. Cancer Treat Rep 61:1623–1629, 1977.
4. Chahinian PA, Arnold DJ, Cohen JM, et al. Chemotherapy for bronchogenic carcinoma. JAMA 237:2392–2396, 1977.
5. Bitran JD, Desser RK, DeMeester TR, et al. Cyclophosphamide, Adriamycin, methotrexate, and procarbazine (CAMP)—effective four-drug combination chemotherapy for metastatic non-oat cell bronchogenic carcinoma. Cancer Treat Rep 60:1225–1237, 1976.
6. Vogelzang NJ, Bonomi PD, Rossof AH, et al. Cyclophosphamide, Adriamycin, methotrexate, and procarbazine (CAMP) treatment of non-oat cell bronchogenic carcinoma. Cancer Treat Rep 62:1595–1597, 1978.
7. Dyke RW, and Nelson RL. Phase I anti-cancer agents. Vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135–142, 1977.
8. Young CW, Currie V, Cvitkovic E, et al. Early phase II evaluation of vindesine in patients with advanced cancer. Curr Chemother 2:1330–1331, 1978.
9. Frei E III, Franzino A, Shnider BI, et al. Clinical studies of vinblastine. Cancer Chemother Rep 12:125–129, 1961.
10. Wright TL, Hurley J, Korst DR, et al. Vinblastine in neoplastic disease. Cancer Res 23:169–179, 1963.
11. Cooperative Study. Neoplastic disease–treatment with vinblastine. Arch Intern Med 116:846–852, 1965.
12. Holland JF, Scharlau C, Gailani S, et al. Vincristine treatment of advanced cancer: a cooperative study of 392 cases. Cancer Res 33:1258–1264, 1973.
13. Sweeney MJ, Boder GB, Cullinan GJ, et al. Antitumor activity of deacetyl vinblastine amide sulfate (vindesine) in rodents and mitotic accumulation studies in culture. Cancer Res 38:2886–2891, 1978.
14. Todd GC, Gibson WR, and Morton DM. Toxicology of vindesine (desacetyl vinblastine amide) in mice, rats, and dogs. J Toxicol Environ Health 1:843–850, 1976.
15. Ochs S, and Worth R. Comparison of the block of fast axoplasmic transport in mammalian nerve by vincristine, vinblastine and desacetyl vinblastine amide sulfate (DVA). Proc Am Assoc Cancer Res and ASCO 16:70, 1975.
16. Bodey GP, and Freireich EJ. Initial clinical studies of vindesine (desacetyl vinblastine amide sulfate). Proc Am Assoc Cancer Res and ASCO 17:128, 1976.
17. Currie VE, Wong PP, Krakoff IH, et al. Phase I trial of vindesine in patients with advanced cancer. Cancer Treat Rep 62:1333–1336, 1978.
18. Smith IE, Hedley DW, Powles TJ, et al. Vindesine: a phase II study in the treatment of breast carcinoma, malignant melanoma, and other tumors. Cancer Treat Rep 62:1427–1433, 1978.
19. Kelsen DP, Bains M, Golbey R, et al. Vindesine in the treatment of esophageal carcinoma. Proc Am Assoc Cancer Res and ASCO 20:338, 1979.
20. Reynolds TF, Cvitkovic E, Golbey R, et al. Phase II trial of vindesine (DVA) in patients with germ cell tumors. Proc Am Assoc Cancer Res and ASCO 20:338, 1979.
21. Mathé G, Misset JL, De Vassal F, et al. Phase II clinical trial with vindesine for remission induction in acute leukemia, blastic crisis of chronic myeloid leukemia, lymphosarcoma, and Hodgkin's disease: absence of cross-resistance with vincristine. Cancer Treat Rep 62:805–809, 1978.
22. Robertson JH, and McCarthy GM. Periwinkle alkaloids and the platelet count. Lancet 2:353–355, 1969.
23. Livingston RB, and Carter SK. Single Agents in Cancer Chemotherapy. New York, NY, IFI/Plenum, 1970, pp 279–297.
24. Brugarolas A, Lacave AJ, Ribas A, et al. Vincristine (NSC 67574) in non-small cell bronchogenic carcinoma. Results of a phase II clinical study. Eur J Cancer 14:501–505, 1978.

Long-Term Survivorship in Small-Cell Anaplastic Lung Carcinoma [1]

Brief Reports and Preliminary Communications

Sandra J. Ginsberg,* Robert L. Comis, Arlan J. Gottlieb, Gerald B. King, Jack Goldberg, Kenneth Zamkoff, Ahmad Elbadawi, and John A. Meyer [2]

Over the past 10 years the prognosis of small-cell anaplastic lung cancer has been radically altered. Developments in chemotherapy have transformed this disease from an almost uniformly and rapidly fatal malignancy to a cancer in which complete responses to chemotherapy (1,2) and, consequently, prolongation of survival (3–6) may be observed.

Between October 1974 and April 1977, 72 patients with newly diagnosed small-cell anaplastic carcinoma were treated at our institution with three different combination chemotherapy and radiotherapy programs. Nine of these 72 patients achieved prolonged disease-free survival, of > 24 months in duration. This paper describes the three therapeutic programs used and the characteristics of the nine long-term survivors.

METHODS

Between October 1974 and April 1977, all patients with newly diagnosed small-cell anaplastic carcinoma in our institution were considered for therapy with three successive combined-modality programs. Patients were excluded from the programs only if they refused to give informed consent, they would not be geographically accessible for followup, or they had an absolute contraindication to one of the chemotherapeutic agents.

There were 52 males among our 72 patients with small-cell anaplastic carcinoma. Their ages ranged from 43 to 67 years with a median age of 56 years. Diagnosis in each case was based upon either histologic or cytologic specimens, which were reviewed by a reference pathologist.

All patients underwent pretreatment staging consisting of the evaluation of liver, bone, and brain by radionuclide scans, bilateral iliac crest bone marrow aspirates and biopsies, and liver function tests. Computerized axial tomography of the brain was used when the radionuclide brain scan was negative for metastatic disease but the patient had signs or symptoms referrable to the central nervous system.

These staging procedures identified 36 patients with clinically limited disease (disease confined to one hemithorax and the ipsilateral supraclavicular nodes) and 36 patients with clinically extensive disease (disease beyond one hemithorax and the ipsilateral supraclavicular nodes).

Thirty-nine patients, 20 with limited disease and 19 with extensive disease, received chemotherapy with program A: cyclophosphamide (700 mg/m^2 iv q3weeks), vincristine (2 mg iv q3weeks), and CCNU (70 mg/m^2 orally q6weeks). Radiotherapy, consisting of 2400 rads in eight sessions over a 2-week period to the primary disease and draining nodes, was included in this program for all patients, as previously described (7). Two of the 20 patients with limited disease underwent lobectomy, with removal of all macroscopic disease, at the time of diagnosis.

Twenty-three patients, 12 with limited disease and 11 with extensive disease, received chemotherapy with program B: cyclophosphamide (1000 mg/m^2 iv q3weeks), vincristine (2 mg iv q3weeks), and CCNU (100 mg/m^2 orally q6weeks) alternating every 6 weeks with Adriamycin (75 mg/m^2 iv q3weeks) and vincristine (2 mg iv q3weeks). Patients with limited disease and patients with severe local complications also received a split course of radiotherapy totaling 4500 rads to the primary lesion and draining lymph nodes, as previously described (8). One of the 12 patients with limited disease underwent lobectomy, with removal of all macroscopic disease, at the time of diagnosis.

Ten patients, four with limited disease and six with extensive disease, received therapy with program C: hexamethylmelamine (8 mg/kg/day orally × 21 days q6weeks), Adriamycin (60 mg/m^2 iv q3weeks), and vincristine (2 mg iv q3weeks) alternating every 6 weeks with cyclophosphamide (700 mg/m^2 iv q3weeks), vincristine (2 mg iv q3weeks), and CCNU (70 mg/m^2 orally q6weeks). Radiotherapy, consisting of 2400 rads in eight fractions over 12 days to the primary lesion and its draining lymph nodes, was administered to all patients. This program, which included hexamethylmelamine, was terminated because of inadequate patient compliance due to severe nausea.

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TABLE 1.—Response rates and disease-free survival in each of the treatment programs

	Treatment program			Totals
	A	B	C	
Total No. of patients (No. of patients with limited disease)	39(20)	23(12)	10(4)	72(36)
Total No. of patients with complete response (%)	18(49%)*	10(45%)†	6(60%)	34(49%)
No. of limited-disease patients with complete response (%)	14(78%)*	6(55%)†	3(75%)	23(70%)
Median duration of all complete responses (mos)	14*	12†	21	
Median survival of all complete responders (mos)	16*	18†	26	
No. of patients disease-free at 16–18 mos (%)	5(13%)	2(9%)	2(20%)	9(13%)
No. of limited-disease patients disease-free at 16–18 mos (%)	4(20%)	2(17%)	2(50%)	8(22%)

*2 patients who had lobectomies are not included in response data.
†1 patient who had a lobectomy is not included in response data.

None of the 72 patients received prophylactic cranial irradiation. Chemotherapy in each of the three treatment programs was continued for 16–18 months. The nine patients in complete clinical remission at that time were re-evaluated with repeat liver, brain, and bone scans, bilateral bone marrow aspirates and biopsies, and liver function tests. Six of these nine patients also underwent bronchoscopy with bronchial washings, brushings, and random biopsies, and laparotomy with open-liver biopsy. Open-liver biopsies were performed to assess their value in the detection of small metastatic deposits during re-staging. All therapy was discontinued for the patients whose complete remissions were confirmed by these re-staging procedures. These patients have been observed to determine their unmaintained disease-free survival.

RESULTS

The complete response rates in each program and the percentage of patients in each program who were clinically in continuous complete remission after 16–18 months of therapy are shown in table 1. Two patients with limited disease in program A and one in program B had undergone lobectomy at the time of diagnosis. These patients are included in the total number of patients, but have been excluded from the response data. Nine patients remained clinically free of disease after 16–18 months of therapy. Eight of the nine patients had limited disease at presentation and three of these eight were patients who had undergone lobectomy. One patient who remained disease-free after 18 months of therapy had hepatic metastases, documented by liver biopsy, at presentation.

The disease-free status of each of the nine patients was confirmed by re-staging. After re-staging, therapy was discontinued in each case and the nine patients were observed. Eight patients have been continually disease-free for 24–42 months. The median disease-free interval is 34 months. They have received no therapy for between 8 and 28 months. The median time off therapy is 16 months. The patient who had extensive disease at the time of diagnosis has been disease-free for 40 months and has been off therapy for 22 months. One patient (treated with program A) developed locally recurrent disease plus intracerebral metastases 4 months after therapy was discontinued. This patient had been re-staged with bronchoscopy and open-liver biopsy. No abnormalities were seen on bronchoscopy. Bronchial brushings, washings, and several random biopsies were also negative for malignancy.

A review of the initial histologic specimens of each of the nine patients reveals that three patients had pure oat-cell variant of small-cell anaplastic carcinoma. Six patients had intermediate cell types as follows: oat cell plus polygonal cell, one patient; pure polygonal cell, three patients; and polygonal cell plus spindle cell, two patients.

DISCUSSION

The cases of prolonged disease-free survival in small-cell anaplastic lung carcinoma discussed here and those reported by others raise the possibility of cure in this disease. However, recent data concerning the doubling time of this tumor (9) suggest that a 4–5-year disease-free survival would be required prior to considering a

patient with this tumor to be cured. That late relapses do occur is verified by the one patient who developed recurrent disease after a 20-month disease-free period.

Most patients with small-cell anaplastic carcinoma of the lung who achieve prolonged disease-free survival have had clinically limited disease at the time of diagnosis. However, the patient reported here with liver metastases at diagnosis suggests that prolonged survival may be possible even in the presence of a large disseminated tumor mass.

The role of re-staging prior to discontinuation of therapy in this disease has not been defined. The re-staging procedures used in this study did not reveal any occult disease among those patients who were clinically disease-free after 16–18 months of therapy. Moreover, these procedures failed to identify residual disease in the one patient who relapsed.

The optimal duration of continued chemotherapy in small-cell anaplastic lung carcinoma is unknown. Nevertheless, it seems unlikely from the cases presented here and from other cases in the literature that therapy beyond 18 months contributes to prolonged disease-free survival. Thus, small-cell anaplastic lung carcinoma may be similar to other highly responsive neoplasms in which a proportion of the patient population may achieve prolonged unmaintained remissions.

REFERENCES

1. Bunn PA, Jr, Cohen MH, Ihde DC, et al. Commentary: Advances in small cell bronchogenic carcinoma. Cancer Treat Rep 61:333–342, 1977.
2. Broder LE, Cohen MH, and Selawry OS. Treatment of bronchogenic carcinoma. II. Small cell cancer. Cancer Treat Rev 4:219–260, 1977.
3. Einhorn LH, Bond WH, Hornback N, et al. Long-term results in combined-modality treatment of small cell carcinoma of the lung. Semin Oncol 5:309–313, 1978.
4. Holoye PY, Samuels ML, Lanzotti V, et al. Combination chemotherapy and radiation therapy for small cell carcinoma. JAMA 237:1121–1124, 1977.
5. Israël L, Depierre A, Choffel C, et al. Immunochemotherapy in 34 cases of oat cell carcinoma of the lung with 19 complete responses. Cancer Treat Rep 61:343–347, 1977.
6. Greco A, Einhorn LH, Richardson R, et al. Small cell lung cancer: progress and perspectives. Semin Oncol 5:323–335, 1978.
7. King G, Comis R, Ginsberg S, et al. Combination multiple agent chemotherapy and radiotherapy in small cell carcinoma of the lung. Radiology 125:529–530, 1977.
8. Ginsberg S, King G, Tinsley R, et al. Intensive alternating combination chemotherapy of small cell anaplastic lung cancer. Proc Am Assoc Cancer Res and ASCO 19:377, 1978.
9. Brigham BA, Bunn PA, Minna JD, et al. Growth rates of small cell bronchogenic carcinomas. Cancer 42:2880–2886, 1978.

Six-Drug Combination Chemotherapy for Nonresectable Bronchogenic Carcinoma[1]

Brief Reports and Preliminary Communications

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Carcinoma of the lung has an overall 5-year survival rate of < 10% and a median survival time of 6–8 months for patients with unresectable tumors (1). Prior studies have not shown that treatment of patients with unresectable disease has improved overall survival (2). A number of single-agent and combination-chemotherapy trials have demonstrated objective responses in bronchogenic carcinoma and definite increased survival in responders compared to nonresponders (3–8). This is a report of our results with a six-drug combination-chemotherapy and radiation-therapy study in 36 patients with stage III bronchogenic carcinoma.

MATERIALS AND METHODS

Thirty-six patients with stage III bronchogenic carcinoma were treated in this study. Twenty-six patients had M_1 disease as defined in the TNM classification. All patients had documented histologic proof of lung cancer. There were 29 males and seven females ranging in age from 35 to 74 years (median, 57 years). The distribution of patients by cell type is as follows: 15 squamous cell carcinomas, 14 adenocarcinomas, four large cell carcinomas, and three small cell carcinomas. Performance status (PS) was evaluated in all patients according to the Karnofsky index. The mean and median PSs were 83 and 90, respectively, with only four patients having a PS of < 60. All patients but one had a smoking history of > 20 pack years. All patients were Caucasian except for two black males. Six patients originally had stage I disease and were in relapse when entered in the study. None of the patients had had prior chemotherapy. One of the six patients with recurrent disease had received bacillus Calmette-Guérin for 2 years; he relapsed within 3 months after completing his immunotherapy.

The chemotherapy schedule is listed in table 1 and consisted of an 8-week cycle of sequential chemotherapy with CCNU, cyclophosphamide, Adriamycin, procarbazine, hexamethylmelamine, and methotrexate. After completion of two cycles of chemotherapy, patients underwent radiation therapy to the primary tumor bed (4000–5000 rads) with resumption of chemotherapy at 50% dose levels, and increased to full doses if no hematologic toxicity occurred. Thirteen patients received radiation therapy to their primary lung lesion and one patient received whole-brain irradiation before beginning chemotherapy because they were symptomatic from their primary tumor or the lesion was extremely large (> 4 cm) and centrally located. After completion of their radiation, these 14 patients began chemotherapy at 50% of the scheduled dose levels. Chemotherapeutic responses in these patients were based on residual disease after completion of their radiation therapy.

Patients continued to receive chemotherapy per protocol until obvious evidence of disease progression occurred. Those patients who failed to respond were switched to other therapeutic regimens and continued to be followed for survival; appropriate dose modifications were made for hematologic and liver toxicity. Complete remission (CR) required a complete disappearance of all clinically detectable evidence of tumor; partial remission (PR) required a reduction of at least 50% in the product of the longest perpendicular diameters of evaluable lesions without the progression of any lesion or the appearance of any new lesions.

RESULTS

The overall results of this study are summarized in table 2. Because of the known improved response rate of small cell carcinoma to combination chemotherapy, the three patients with small cell carcinoma are not considered in this evaluation. This leaves a total of 33 evaluable patients.

There were two CRs and four PRs, for an overall response rate of 18%. The six responders survived significantly longer than the 27 nonresponders ($P = 0.001$). The median survival was greatest for patients achieving a CR: 764 days. When all patients who experienced either a CR or a PR are considered, the overall median survival time was 517 days. The median survival of all nonre-

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[3]The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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TABLE 1.—Chemotherapy schedule: 8-wk sequential cycle

Drug	Dose (mg/m^2)	Schedule (days)	Route of administration
CCNU	65	1	oral
Cyclophosphamide	500	1	iv
Adriamycin	30	2	iv
Procarbazine	100	8–18	oral
Hexamethylmelamine	250	8–22	oral
Methotrexate	20	29, 33, 36, and 40	oral

TABLE 2.—Tumor response

	CR	PR	CR + PR	No response
No. of patients (%)	2 (11)	4 (17)	6 (18)	27 (72)
Median duration of survival in days	764	269	517	155

TABLE 3.—Tumor response in patients with M_1 disease

	CR	PR	CR + PR	No response
No. of patients (%)	1 (5)	4 (18)	5 (23)	17 (77)
Median duration of survival in days	450	228	234	145

sponders, including those patients who did not receive one full course of treatment, was significantly shorter: 155 days. Three patients remain alive, one of whom continues in CR at 730 days. All other patients have died from disease progression. Of the 26 patients with M_1 disease entered in the protocol, 22 were considered evaluable. The results in this group are shown in table 3. The median survival time (234 days) of these 22 patients is less than that of the entire group. Including all M_1 patients originally entered in the study, the overall response was five of 26 patients or 19%. Table 4 illustrates the effect of prior radiation on response and survival. Of the 14 patients who received radiation therapy prior to chemotherapy, only 13 were evaluable, and in these patients either no response or only minimal changes occurred with radiation. Only one of these 13 patients showed a PR with subsequent chemotherapy as measured by a decrease in tumor size on chest roentgenogram. No patients who responded to initial chemotherapy had further disease regression with the scheduled radiation therapy.

Toxicity was limited to the gastrointestinal and hematologic systems. Nausea and vomiting occurred in 24 of the 36 patients and was severe enough in four patients to cause them to decline further therapy. Hematologic toxicity was almost exclusively manifested as leukopenia. Platelet counts remained within normal ranges; only three patients had platelet counts of $< 50{,}000/mm^3$. There were no episodes of significant clinical bleeding in any of the 36 patients. Eighteen of 36 patients did develop significant leukopenia with total wbc counts ranging from 800 to $2000/mm^3$ (median, $1500/mm^3$). There were two deaths from sepsis and four other septic episodes requiring hospitalization in patients who were leukopenic.

The mean PS was 95 for responders and only 80 for nonresponders; while this difference is small, it supports the findings of other studies that a higher PS is generally indicative of a better response (3,8).

TABLE 4.—Effect of prior radiation therapy (RT) on response and survival

	CR	PR	No response
		Prior RT	
No. of patients	0	1	12
Median duration of survival in days	—	304	212
		No prior RT	
No. of patients	2	3	15
Median duration of survival in days	764	234	145

DISCUSSION

The results of our study compare favorably with others previously reported but are not significantly better (3,8–10).

In Hodgkin's disease, combinations of drugs with supposedly varying sites of action in the cell cycle will produce increased numbers of tumor cell kill without additive toxicity. In our protocol six different drugs were used in combination based on this theory, yet our results are no better than any other two-, three-, four-, or five-drug combinations previously reported. A response rate of 18% is in fact not superior to results obtained with single-agent chemotherapy (11). The addition of radiation therapy administered either before or during chemotherapy did not appear to influence survival. This suggests that, in non-oat cell bronchogenic carcinoma, presently available agents are not going to achieve objective response rates much greater than 20%–40% and will not meaningfully affect overall survival. In the future, greater emphasis might be placed on newer agents used singly or in combination for the treatment of stage III bronchogenic carcinoma.

REFERENCES

1. SELAWRY OS, and HANSEN HH. Lung cancer. *In* Cancer Medicine (Holland JF, and Frei E III, eds). Philadelphia, Pa, Lea and Febiger, 1973, pp 1473–1518.
2. DURVANT KR, ELLIS F, BLOCK JM, ET AL. Comparison of treatment policies in inoperable bronchogenic carcinoma. Lancet 1:715–719, 1971.
3. ALBERTO P, BRUNNER KW, MARTZ G, ET AL. Treatment of bronchogenic carcinoma with simultaneous or sequential combination chemotherapy, including methotrexate, cyclophosphamide, procarbazine and vincristine. Cancer 38:2208–2216, 1976.
4. RICHARDS F II, COOPER MR, MUSS HB, ET AL. Methyl-CCNU, alone and in combination with vincristine, or vincristine and methotrexate, in advanced bronchogenic carcinoma. Cancer 38:1077–1082, 1976.
5. STRAUS MJ. Combination chemotherapy in advanced lung cancer with increased survival. Cancer 38:2232–2241, 1976.
6. LIVINGSTON RB. Nitrosourea combinations in lung cancer. Cancer Treat Rep 60:757–760, 1976.
7. HANSEN HH, SELAWRY OS, SIMON R, ET AL. Combination chemotherapy of advanced lung cancer. A randomized trial. Cancer 38:2201–2207, 1976.
8. BITRAN JD, DESSER RK, DEMEESTER TR, ET AL. Cyclophosphamide, Adriamycin, methotrexate, and procarbazine (CAMP)—effective four-drug combination chemotherapy for metastatic non-oat cell bronchogenic carcinoma. Cancer Treat Rep 60:1225–1230, 1976.
9. CHAHINIAN AP, ARNOLD DJ, COHEN JM, ET AL. Chemotherapy for bronchogenic carcinoma. JAMA 237:2392–2396, 1977.
10. SAMSON MK, BAKER LH, FRAILE RJ, ET AL. Combination chemotherapy with cyclophosphamide, vincristine, methyl-CCNU, and bleomycin in advanced bronchogenic carcinoma: experience with 106 patients. Cancer Treat Rep 61:59–64, 1977.
11. CARBONE PP, FROST JK, FEINSTEIN AR, ET AL. Lung cancer: perspective and prospects. Ann Intern Med 73:1003–1024, 1970.

Phase II Evaluation of Diglycoaldehyde, VP-16-213, and the Combination of Methyl-CCNU and β-2′-Deoxythioguanosine in Previously Treated Patients With Colorectal Cancer: An Eastern Cooperative Oncology Group Study (EST-1275)[1]

Brief Reports and Preliminary Communications

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SUMMARY

The Eastern Cooperative Oncology Group assessed the activity of diglycoaldehyde (DGA), VP-16-213, and the combination of methyl-CCNU and β-2′-deoxythioguanosine in previously treated patients with advanced colorectal cancer. Objective responses were seen in two of 40 evaluable patients receiving methyl-CCNU and β-2′-deoxythioguanosine and in one of 35 patients receiving DGA. None of 33 patients responded to VP-16-213, but one death related to sepsis and bone marrow failure occurred. Survival of patients whose previous chemotherapy included a nitrosourea was markedly shortened compared to those who had not been exposed to nitrosoureas. With the possible exception of DGA, further treatment of patients with colorectal cancer with these therapies is not warranted.

[Cancer Treat Rep 63:1355–1357, 1979]

Lack of effective chemotherapy for patients with previously treated metastatic colorectal cancer led the Eastern Cooperative Oncology Group (ECOG) to search for activity among phase II drugs and drug combinations. Diglycoaldehyde (DGA) showed modest activity in patients with a variety of solid tumors and showed some activity against colorectal cancer in a phase I trial (1). VP-16-213 was found to be active against breast cancer, liposarcoma, and Wilms' tumor (2), but had not been used in colorectal cancer. The combination of methyl-CCNU (MeCCNU) and β-2′-deoxythioguanosine (BTG), each of which were derivatives of drugs that had shown some activity in colorectal cancer (3), was proposed in 1974 in a search for increased therapeutic benefit. MeCCNU later demonstrated antineoplastic activity against colorectal cancer (4), while BTG did not.

MATERIALS AND METHODS

One third of 190 patients randomized between July 1975 and August 1976 were ineligible for treatment, largely because of persisting effects of previous chemotherapy. Thus, 108 patients were fully evaluable in the initial treatment study and 41 were evaluable in the crossover phase. A total of 40 evaluable patients received MeCCNU (130 mg/m^2 orally on Day 1) and BTG (60 mg/m^2 iv on Days 1–5 and 29–33) on an 8-week cycle, while 33 received VP-16-213 (125 mg/m^2 iv on Days 1, 3, and

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5, q4weeks) and 35 received DGA (fully ambulatory patients [2 g/m^2] versus not fully ambulatory patients [1.5 g/m^2] iv on Days 1–3, q4weeks). Within the protocol, 19 patients crossed-over to secondary therapy with VP-16-213 and 22 crossed-over to DGA. Prior to treatment, a fully informed consent was obtained.

Of 55 patients previously treated with 5-fluorouracil only, 40 received the MeCCNU–BTG combination, eight were treated with VP-16-213, and seven were treated with DGA. Of the 53 patients previously treated with nitrosoureas 25 received VP-16-213 and 28 received DGA.

All patients had histologically confirmed measurable metastatic disease, met baseline hematologic minimum values, were neither azotemic nor jaundiced, and had not been treated with other drugs within 4 weeks. Forty percent of the total patients were referred from the Roswell Park Memorial Institute. Patients who had received radiation therapy (> 4000 rads) were treated at 25%–33% dose reductions of protocol drugs during the first treatment course.

Partial response in patients with hepatomegaly as the indicator lesion was defined as a reduction of ≥ 30% in the sum of liver measurements below the costal margins at the midclavicular lines and in the subxiphoid region, which was accompanied by improvement in abnormalities of liver function (4). For hepatomegaly to be measurable, the liver had to be palpable ≥ 5 cm below the costal margin during quiet respiration.

Survival was measured as the time between initiation of chemotherapy on this protocol and death.

RESULTS

Severe to life-threatening toxic effects (table 1)occurred in 14 patients given MeCCNU plus BTG: two were due to intractable vomiting and 12 were due to hematologic effects (anemia, thrombocytopenia, or leukopenia). One of eight patients with severe or life-threatening hematologic toxicity after receiving VP-16-213 died with sepsis, bleeding, and hepatic failure. Vomiting was severe in seven patients given DGA. There was severe bone marrow depression in one of these patients and in two other patients, but no patient given DGA was reported to have life-threatening toxicity. Hypotension, reported as a complication of DGA therapy (1), was controlled by infusion of the drug in a buffered dilute solution over 1 hour and by monitoring blood pressure and pulse at 10-minute intervals.

TABLE 1.—ECOG toxicity criteria

Parameter evaluated	Toxicity criteria	
	Severe	Life-threatening
Leukopenia	Wbc count, 1999–10,000/mm^3	Wbc count, < 1000/mm^3
Thrombocytopenia	Platelet count, 25,000–49,999/mm^3	Platelet count, < 25,000/mm^3
Anemia	Requires transfusion	
Gastrointestinal toxicity	Intractable vomiting, bloody diarrhea	

There were three objective partial responses noted in the entire series: two from MeCCNU plus BTG lasting 10 and 14 months and one from DGA (the patient had been previously treated with a nitrosourea) lasting 5 months. In all three patients, hepatomegaly was the indicator lesion and liver metastases were the site of tumor regression. There were no responders to cross-over treatment.

Differences in survival associated with the three treatment programs under study were not significant. Median survival times were as follows: VP-16-213, 18 weeks; DGA, 22 weeks; and MeCCNU plus BTG, 24 weeks. The overall median survival time of 21 weeks was consistent with previous group studies among patients with advanced measurable colorectal cancer who were previously treated.

The major finding of this study was the effect of prior chemotherapy on survival. Patients entered this study with prior 5-fluorouracil or nitrosourea therapy. Patients previously treated with 5-fluorouracil lived significantly longer than patients previously treated with nitrosoureas (25 versus 19 weeks, $P < 0.02$). Correcting for imbalances in the patient population (5), the observed difference of 6 weeks becomes an adjusted difference of 10 weeks between these two previously treated subgroups. Table 2 shows the actual median survival times for the three treatment programs according to prior therapy.

The median survival time of the ambulatory (ECOG performance status 0–1) group was 14 weeks longer than that of the semiambulatory (ECOG performance status 2–3) group (27 versus 13 weeks, $P < 0.0001$). The median survival of patients with liver involvement was significantly less than that of patients without liver involvement at the time of randomization into this protocol (16 versus 27 weeks, $P < 0.0001$). Those patients in whom protocol therapy was instituted > 6 months after the original diagnosis of cancer lived 23 weeks after beginning protocol therapy, whereas those with a shorter interval survived for only 16 weeks.

DISCUSSION

Previous ECOG experience in advanced colon and rectal cancer (4) had shown no survival advantage for patients whose initial antineoplastic chemotherapy included a nitrosourea compared to patients whose initial chemotherapy did not. Thus, total survival of patients with advanced colorectal cancer does not appear to have been

TABLE 2.—Median survival time according to prior chemotherapy—EST–1275 treatment programs

Prior chemotherapy	Median survival time in wks			
	MeCCNU + BTG	VP–16–213	DGA	Totals
5-Fluorouracil	24	19	42	25
Nitrosoureas	—	17	20	19
Totals	24	18	22	21

prolonged by nitrosourea therapy. The significantly shorter survival of patients treated with this protocol who had previously received a nitrosourea would suggest that these patients are poor candidates for most further chemotherapeutic trials.

Although few previously treated patients can be expected to respond to secondary treatment programs, the survival of those few responders can be markedly prolonged. Were it not for the subsequent ECOG experience (6) suggesting that a response rate approximating that usually reported in previously untreated patients can be demonstrated in patients with colorectal cancer who had been previously treated with 5-fluorouracil, these responses could suggest the discontinuation of further drug studies in previously treated patients.

The results of the present trial would suggest that further evaluation of VP-16-213 and the MeCCNU plus BTG combination should not be undertaken in previously untreated patients with colorectal cancer, while DGT, which was not totally inactive, might be considered in a limited number of untreated patients.

REFERENCES

1. KAUFMAN JH, and MITTELMAN A. Phase I study of inosine dialdehyde (diglycoaldehyde, NSC 118994). Proc Am Assoc Cancer Res and ASCO 16:51, 1975.
2. CARTER SK, and SLAVIK M. Investigational drugs under study by the United States National Cancer Institute. Cancer Treat Rev 3:43–60, 1976.
3. HORTON J, MITTELMAN A, TAYLOR SG III, ET AL. Phase II trials with procarbazine (NSC-77213), streptozotocin (NSC-85998), 6-thioguanine (NSC-752), and CCNU (NSC-79037) in patients with metastatic cancer of the large bowel. Cancer Chemother Rep 59:333–340, 1975.
4. DOUGLASS HO, JR, LAVIN PT, WOLL J, ET AL. Chemotherapy of advanced measurable colon and rectal carcinoma with oral 5-fluorouracil, alone or in combination with cyclophosphamide or 6-thioguanine, with intravenous 5-fluorouracil or beta-2'-deoxythioguanosine or with oral 3(4-methyl-cyclohexyl)-1(2-chlorethyl)-1-nitrosourea. A phase II–III study of the Eastern Cooperative Oncology Group (EST 4273). Cancer 42:2538–2545, 1978.
5. Cox DR. Regression models in life tables. J R Stat Soc 34:187–220, 1972.
6. ENGSTROM P, MACINTYRE J, DOUGLASS H, JR, ET AL. Combination chemotherapy of advanced bowel cancer. Proc Am Assoc Cancer Res and ASCO 19:384, 1978.

Phase II Evaluation of Hexamethylmelamine in Advanced Breast Cancer: A Southwest Oncology Group Study [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Ninety-eight patients with metastatic breast cancer, heavily pretreated with other agents, were entered in a phase II trial of hexamethylmelamine (HEX). Patients were randomized to receive HEX alone or combined with prophylactic pyridoxine. There was a 2% response rate in 89 partially or fully evaluable patients. Seven percent of these patients developed neurologic toxicity which occurred in the HEX-alone group only.

[Cancer Treat Rep 63:1359–1361, 1979]

Hexamethylmelamine (HEX) is a triazine derivative which may function as an antimetabolite although it was initially thought to be an alkylating agent (1). In early broad phase II trials, response rates of 13%–23% were reported in small numbers of patients with breast cancer, using dose regimens varying from 8 mg/kg/day continuously to 12 mg/kg/day for 21 days (2–4). Dose-limiting toxicity in these early trials consisted of nausea, vomiting, and central and peripheral neurologic symptoms (2–10). Severe peripheral neuropathy was more commonly seen when patients had received the drug for ≥ 5 months (11). It appeared to be reversible with drug withdrawal. The ability to continue receiving the drug after the onset of neuropathy with reduction in drug dosage and the addition of pyridoxine has been reported anecdotally (11). The present trial was undertaken to determine the response to HEX in patients with metastatic breast cancer refractory to standard therapeutic agents. In addition, patients were randomized to receive or not to receive prophylactic pyridoxine to determine whether or not this could decrease the incidence of neurotoxicity.

MATERIALS AND METHODS

Ninety-eight patients with advanced breast cancer were entered in this phase II trial. Patient eligibility requirements included: (*a*) histologically proven breast cancer; (*b*) tumor resistant to known effective agents; (*c*) measurable disease; (*d*) no concomitant therapy; and (*e*) no prior hormonal therapy received for 3 weeks before the study (patients with clearly progressive disease) or for 6 weeks (patients with stable disease).

Patients were classified as good- or poor-risk patients; within these two groups they were randomized to receive HEX with pyridoxine (100 mg orally tid) or without pyridoxine. Poor-risk patients were defined as those with any of the following characteristics: (*a*) > 65 years of age; (*b*) wbc count of < 4000/μl or platelet count of < 150,000/μl; (*c*) prior intolerance of chemotherapy; (*d*) prior nitrosourea therapy; or (*e*) prior radiation therapy given to ≥ 50% of the bone marrow-bearing areas. All other patients were classified as good-risk patients. Thus, there were four treatment groups: *treatment 1*—good-risk patients receiving HEX at a dose of 320 mg/m^2/day for 21 days + pyridoxine at a dose of 100 mg tid; *treatment 2*—good-risk patients receiving HEX at a dose of 320 mg/m^2/day for 21 days; *treatment 3*—poor-risk patients receiving HEX at a dose of 240 mg/m^2/day for 21 days + pyridoxine at a dose of 100 mg tid; and *treatment 4*—poor-risk patients receiving HEX at a dose of 240 mg/m^2/day. Treatment courses were repeated every 6 weeks if the patient had recovered from the toxicity of the previous course.

Patients were evaluated before treatment with cbc, platelet count, liver and renal function tests, roentgenograms of chest, spine, pelvis, and any other involved or painful areas, and liver scan. Blood cell counts were repeated on Days 1, 21, and 28 of each 6-week cycle. Assessment of tumor response was performed every 6 weeks. Complete response was defined as the disappearance of all known disease for at least 4 weeks, partial

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TABLE 1.—% of patients developing toxicity*

Degree	Wbc count/μl	Platelet count/μl	Gastrointestinal toxicity	Neurologic toxicity
None	≥4000 (79%)	≥100,000 (81%)	33%	93%
Mild	3000–4000 (11%)	75,000–100,000 (9%)	16%	2%
Moderate	2000–3000 (8%)	50,000–75,000 (9%)	22%	2%
Severe	<2000 (2%)	<50,000 (1%)	29%	2%

*Based on 89 fully or partially evaluable patients for hematologic and gastrointestinal toxicity and 88 patients for neurologic toxicity.

response was defined as a decrease of ≥ 50% in the sum of the product of diameters of measurable lesions for at least 4 weeks, and improvement was defined as a decrease of < 50% but ≥ 25% of the sum of the product of tumor diameters. Lytic bone lesions, if present, must have shown evidence of reossification for the patient to be classified as having a complete or partial response.

Ninety-eight patients were entered in the study. Nine patients were not evaluable. The original goal was to enter 25 evaluable patients in each of the four treatment groups to allow estimation of response with a standard of ± 10%. Entry was stopped slightly short of this goal because of the dearth of responses. Eighty-nine patients were fully or partially evaluable: 12 in treatment 1, 26 in treatment 2, 20 in treatment 3, and 31 in treatment 4 as defined above. Fully evaluable patients were those who completed 3 weeks (one course) of HEX. Partially evaluable patients were those who began HEX treatment but for some reason did not complete the initial 3-week course. There were 25 partially evaluable patients; six of these died within 14 days from the beginning of treatment. All early deaths were from tumor. Sixteen patients (18% of the study population) had an inadequate trial resulting from toxicity. One patient developed lymphangitic pulmonary tumor spread after receiving treatment for a few days, and was switched to another regimen. Two patients who had no documented toxicity refused further treatment. All patients were heavily pretreated. Of the 89 fully or partially evaluable patients, 85% had had prior Adriamycin, cyclophosphamide, methotrexate, and 5-fluorouracil, and 37% had had prior vincristine. The median performance status was 2 (ambulatory but symptomatic).

RESULTS

There were two partial responses lasting 16 and 16+ weeks and two improvements lasting 4 weeks each. The partial response rate was 3% (two responses among 65 patients) of the fully evaluable patients and 2% (two responses among 89 patients) of the fully and partially evaluable patients. All partial responses and improvements occurred in patients with soft tissue predominant disease. Both partial responses and one of the improvements occurred using the high-dose HEX-alone arm. The partial response rate for this arm was 8% (two responses among 26 patients).

The median survival of all fully or partially evaluable patients was 11 weeks. Survival times for partial responses and improvements were 20+, 16+, 10, and 8+ weeks.

Toxicity data are based on the 89 partially or fully evaluable patients. The major toxic effects were nausea and vomiting which occurred in 67% of the patients, usually within a few days of starting the drug. Fourteen percent of the patients were unable to continue treatment because of these side effects. Hematologic toxicity was not dose-limiting. Only 16% of the patients developed more than minimal hematologic toxicity. Nadirs were generally seen at 3–4 weeks from the start of the drug. There was no significant difference in the incidence or severity of gastrointestinal or hematologic toxicity between patients receiving high- and low-dose HEX or between those receiving and those not receiving prophylactic pyridoxine. Results are shown in table 1.

Only six (7%) of the 88 patients evaluable for neurotoxicity developed this side effect. All cases occurred in the treatment arms without prophylactic pyridoxine. Results are shown in table 2. The difference in the incidence of neurotoxicity between all patients who received pyridoxine and those who did not was not significant ($0.1 < P < 0.25$). In two patients the toxicity consisted of paresthesias. In four patients neurologic toxicity was moderate to severe and consisted of sensory-motor neuropathies (three patients), severe confusion (one patient), and vertigo (one patient). All four patients with moderate or worse toxicity developed their symptoms within 3 weeks of starting therapy, and two of the four had had prior vincristine.

DISCUSSION

HEX does not appear to be an effective agent in heavily pretreated patients with metastatic breast cancer. The 2% response rate (two responses among 89 patients) is similar to that (no responses among 19 patients) reported in a recent phase II study with similar patient characteristics (12). Dose-limiting toxic effects were nausea and vomiting which occurred in 67% of the patients and re-

TABLE 2.—Incidence of neurologic toxicity in fully and partially evaluable patients*

	High dose: treatment group—		Low dose: treatment group—	
	1	2	3	4
All patients	0/12†	5/26 (19%)	0/20	1/30 (3%)†
Prior vincristine	0/5	2/8 (25%)	0/11	0/8
No prior vincristine	0/7	3/18 (16%)	0/9	1/22 (4.5%)
Without pyridoxine (treatment groups 2 and 4), 6/56 (11%)				
With pyridoxine (treatment groups 1 and 3), 0/32				
Prior vincristine, 2/32 (6%)				
No prior vincristine, 4/56 (7%)				

*1 patient was deleted from neurotoxicity analysis because she was randomized to receive no pyridoxine but was receiving 50 mg/day as part of antituberculous treatment.
†No. of patients with neurotoxicity/No. of patients evaluable.

quired permanent discontinuation (even after dose reduction) in 14%. It is possible that different response results might be obtained if a parenteral form of the drug were available, so that large amounts might be administered on an intermittent basis.

Neurotoxicity occurred in only 7% of the patients. Interestingly, *all* patients who developed neurotoxicity were those not receiving the prophylactic pyridoxine. The incidence of neurotoxicity in patients not receiving pyridoxine was 11%. There was no difference in the incidence of prior vincristine administration between patients who developed neurotoxicity and those who did not, although extremely small numbers developed neurotoxicity. However, in the high-dose regimen without pyridoxine, two (25%) of eight patients with prior vincristine developed neurotoxicity as opposed to none of five patients with prior vincristine who did receive prophylactic pyridoxine. The difference in the incidence of neurotoxicity between patients who received prophylactic pyridoxine and those who did not was not significant ($0.1 < P < 0.25$).

REFERENCES

1. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine: an evaluation of its role in the therapy of cancer. Cancer 38:27–35, 1976.
2. WAMPLER GL, MELLETTE SJ, KUPERMINC M, ET AL. Hexamethylmelamine (NSC-13875) in the treatment of advanced cancer. Cancer Chemother Rep 56:505–514, 1972.
3. WILSON WL, BISEL HF, COLE D, ET AL. Prolonged low-dosage administration of hexamethylmelamine (NSC 13875). Cancer 25:568–570, 1970.
4. WILSON WL, SCHROEDER JM, BISEL HF, ET AL. Phase II study of hexamethylmelamine (NSC 13875). Cancer 23:132–136, 1969.
5. STOLINSKY DC, and BATEMAN JR. Further experience with hexamethylmelamine (NSC-13875) in the treatment of carcinoma of the cervix. Cancer Chemother Rep 57:497–499, 1973.
6. TAKITA H, and DIDOLKAR MS. Effect of hexamethylmelamine (NSC-13875) on small cell carcinoma of the lung (phase II study). Cancer Chemother Rep 58:371–374, 1974.
7. WILSON WL, and DE LA GARZA JG. Phase I study of hexamethylmelamine (NSC-13875). Cancer Chemother Rep 48:49–52, 1965.
8. LOUIS J, LOUIS NB, LINMAN JW, ET AL. The clinical pharmacology of hexamethylmelamine: a phase I study. Clin Pharmacol Ther 8:55–64, 1967.
9. STOLINSKY DC, BOGDON DL, SOLOMON J, ET AL. Hexamethylmelamine (NSC-13875) alone and in combination with 5-(3, 3-dimethyltriazeno)imidazole-4-carboxamide (NSC-45388) in the treatment of advanced cancer. Cancer 30:654–659, 1972.
10. BERGEVIN PR, TORMEY DC, and BLOM J. Clinical evaluation of hexamethylmelamine (NSC-13875). Cancer Chemother Rep 57:51–58, 1973.
11. SMITH JP, and RUTLEDGE FN. Random study of hexamethylmelamine, 5-fluorouracil, and melphalan in the treatment of advanced carcinoma of the ovary. Natl Cancer Inst Monogr 42:169–172, 1975.
12. DENEFRIO JM, and VOGEL CL. Phase II study of hexamethylmelamine in women with advanced breast cancer refractory to standard cytotoxic therapy. Cancer Treat Rep 62:173–175, 1978.

Clinical Trial of Weekly Pyrazofurin [1,2]

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SUMMARY

Pyrazofurin was administered to 21 patients with solid tumors at a dose of 200 mg/m² iv weekly, because this dose had been shown to be well-tolerated and pharmacologic effects of a single dose at this level persisted for up to 7 days. An anemia consistent with a disturbance in rbc production was seen in most patients. Other toxic effects included stomatitis, rash, and myelosuppression. No complete or partial responses were noted, but two patients with alveolar cell carcinoma of the lung each had stable disease for 12 months. Most of the patients in this study tolerated the weekly dosage schedule well with only minimal myelosuppression, suggesting that this agent and schedule might be acceptable for use in combination chemotherapy. Several theoretic reasons favor the use of pyrazofurin in this manner. Pyrazofurin should also be evaluated more fully in patients with polycythemia vera, mycosis fungoides, and psoriasis, since other orotidylate decarboxylase inhibitors have been shown to be effective in these diseases.

[Cancer Treat Rep 63:1363–1365, 1979]

Pyrazofurin is a nucleoside fermentation product of *Streptomyces candidus* (1). The parent compound is activated in vivo to its 5′-phosphate nucleotide derivative. This nucleotide of pyrazofurin has been shown to be a potent inhibitor of orotidylate decarboxylase ($k_i = 5 \times 10^{-9}$ M), the enzyme which catalyzes the conversion of orotidylic to uridylic acid (2). Studies with rat liver have shown that this agent is 20 times more potent an inhibitor of this enzyme than is 6-azauridine, another compound whose target site of action is the same enzyme (3).

Experimentally, pyrazofurin used alone has shown antitumor activity against Walker 256 carcinosarcoma, adenocarcinoma Ca755, Gardner and Mecca lymphosarcomas, X5563 plasma cell myeloma, and C3H mammary carcinoma, but little or no activity against murine leukemias (4). Phase I clinical trials designed to determine tolerable dosage schedules have recently been reported for pyrazofurin (5,6). The dosage schedules studied have included 20–100 mg/m² given daily for 5 days at 3-week intervals, 100–300 mg/m² administered as single doses one to two times per month, and continuous infusions ranging from 250 mg/m²/24 hours to 1500 mg/m²/144 hours. This report describes results seen in 21 patients with solid tumors given pyrazofurin at a fixed dose schedule of 200 mg/m² iv weekly. This dose schedule was chosen because it had been shown to be well-tolerated and pharmacologic effects of a single dose at this level persisted for up to 7 days (7). Thus, it seemed reasonable to administer it at weekly intervals.

MATERIALS AND METHODS

Twenty-one patients with advanced metastatic carcinomas were admitted to this study if they had measurable disease, an Eastern Cooperative Oncology Group (ECOG) performance status of < 4, a peripheral blood leukocyte count of $\geq 4000/mm^3$, and a platelet count of $> 100{,}000/mm^3$. They could not have received any prior chemotherapy for at least 4 weeks, and they were required to give informed consent. The diagnoses included carcinoma of the lung (nine patients), colon (nine patients), breast (two patients), and pancreas (one patient). Eighteen of the patients had failed previous chemotherapy trials and three had unresectable primary tumors (two with non-oat cell lung cancer and one with pancreatic cancer) at the time of entry in the study. There were 13 males and eight females. The median age of the patients was 58 years (range, 37–73).

Pyrazofurin was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md, as a lyophilized powder and was reconstituted with sterile water. It was administered to all patients as an iv bolus

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TABLE 1.—Patient characteristics, response, and toxicity

Type of carcinoma	No. of patients	Age range in yrs	No. of cycles (range)	Response		ECOG toxicity grade (No. of patients)			
				No change	Progressive disease	Stomatitis	Rash	Leukopenia	Thrombocytopenia
Lung	9	37–62	2–56	2*	7	1(2)	1(1)		
						2(1)			2(1)
						3(1)			3(1)
Colon	9	55–66	2–15		9	1(1)	1(1)		
						2(3)	2(1)	2(2)	2(2)
						3(1)	3(1)		
Breast	2	49–66	2–3		2		1(1)		
						2(1)			
						3(1)			
Pancreas	1	73	5		1			2(1)	

*Both alveolar cell type.

at a dose of 200 mg/m^2 once weekly, until the onset of progressive disease or intolerable toxicity precluded further treatment. Fifty-percent dose reductions were made in the presence of mild stomatitis and myelosuppression with leukocyte counts of $< 4000/mm^3$ and/or platelet counts of $< 75,000/mm^3$. ECOG response and toxicity criteria were used to assess the clinical activity of pyrazofurin in all patients studied (8).

RESULTS

Nineteen patients received two to 15 doses (median, 5.4) of pyrazofurin. All patients receiving a minimum of two doses were considered evaluable for response and toxicity. This is summarized in table 1. No complete or partial responses were noted. Two patients with alveolar cell carcinoma of the lung were classified as no change, according to ECOG response criteria. Each of these patients had stable disease for a period of 12 months. They received a total of 43 and 56 doses of pyrazofurin respectively.

Toxicity consisted of anemia (16 patients), stomatitis (11 patients), rash (seven patients), and minimal myelosuppression (five patients). Sixteen of the 21 patients experienced a drop in their hemoglobin level; three had no change, and two required rbc transfusions for gastrointestinal blood loss and severe anemia that were present before treatment with pyrazofurin began. The median fall in hemoglobin due to pyrazofurin was 3.2 g/100 ml, with a range of 0.5–6.2 g/100 ml. This was seen as early as the second week of treatment in some patients and as late as 15 weeks in others. Two thirds of the patients however, reached their lowest hemoglobin level by 7 weeks. The anemia was normochromic, normocytic, and consistent with a disturbance in rbc production. Three patients developed a peripheral blood leukocyte count of $< 3000/mm^3$, but no counts fell to $< 2000/mm^3$. Only three patients had platelet counts of $< 75,000/mm^3$. Two patients developed a generalized cutaneous eruption over the trunk and extremities following the second dose of pyrazofurin. This rash consisted of groups of small red papules and pustules, some with crusts, over a diffuse erythematous base. It did not require stopping treatment in either patient. However, severe stomatitis caused therapy to be interrupted in five patients and stopped in two patients after two doses; it was most prominent in severely cachectic patients.

DISCUSSION

This clinical study of pyrazofurin differs from previously reported studies in that a fixed weekly dose of 200 mg/m^2 iv was administered to all patients. This is in contrast to the 5-day treatment schedule (20–100 mg/m^2) and single higher doses (100–300 mg/m^2), both given to solid tumor patients every 2–3 weeks in phase I evaluations (5,6). Some antitumor activity has been noted in patients with carcinoma of the breast, colon, lung, and kidney in previous trials. In the present study, using a weekly iv bolus administration schedule, no antitumor responses were seen. Two patients did have stable disease, in each case lasting for a period of 12 months. Interestingly, they were the only patients treated who had the diagnosis of alveolar cell carcinoma of the lung. Thus, it might be worthwhile to evaluate this agent further in this particular histologic type of lung cancer. No responses to pyrazofurin treatment have been seen in phase II trials in advanced colorectal carcinoma, visceral-dominant malignant melanoma, and refractory acute myelogenous leukemia using other dosage schedules (9–11), or in patients with lung cancer or bone or soft tissue sarcomas treated weekly (12,13).

The majority of patients tolerated this weekly dosage schedule well with only minimal myelosuppression, sug-

gesting that this agent and schedule might be acceptable for use in combination chemotherapy. There are several theoretic reasons to consider its use this way. A single iv dose at this level has been shown to block orotic acid metabolism in man for a period of up to 7 days (7,14). Furthermore, pyrazofurin given before cytosine arabinoside results in a tenfold increase in acute cell death of cultured leukemic cells over that seen with the reverse sequence of administration (14). These findings are consistent with results noted previously by investigators at our institution who treated L5178Y cells with 6-azauridine, another orotidylate decarboxylase inhibitor, before cytosine arabinoside (15). It has also been suggested that the therapeutic effects of pyrimidine analogs might be enhanced by combining these agents with pyrazofurin (16). Additional preclinical studies would seem warranted before initiating combination chemotherapy trials, however, in view of the disappointing results seen when this agent is used alone.

The development of anemia in the majority of patients in our study is not surprising, in view of the frequency with which this occurs in patients being treated with other orotidylate decarboxylase inhibitors, such as 6-azauridine and triacetylazauridine. In fact, these agents have been shown to be effective in the treatment of patients with polycythemia vera (17). These findings suggest that further studies to evaluate the role of pyrazofurin in the treatment of polycythemia vera appear indicated.

Further studies in patients with psoriasis and mycosis fungoides also are warranted, since orotidylate decarboxylase inhibitors have been shown to be effective in these two conditions as well (18,19).

REFERENCES

1. Williams RH, Gerzon K, Hoehn M, et al. Pyrazofurin, a novel carbon-linked nucleoside. (Abstr MICR 38) *In* Proceedings of the 158th American Chemical Society National Meeting, New York, NY, 1969.
2. Gutowski GE, Sweeney MJ, DeLong DC, et al. Biochemical and biological effects of pyrazofurins (pyrazomycins)—initial clinical trial. Ann NY Acad Sci 255:544–551, 1975.
3. Eli Lilly and Co. Pyrazofurin Information for Clinical Investigators. Indianapolis, Ind.
4. Sweeney MJ, Davis FA, Gutowski GE, et al. Experimental antitumor activity of pyrazomycin. Cancer Res 33:2619–2623, 1973.
5. Salem PA, Bodey GP, Burgess MA, et al. A phase I study of pyrazofurin. Cancer 40:2806–2809, 1977.
6. Ohnuma T, and Holland JF. Initial clinical study with pyrazofurin. Cancer Treat Rep 61:389–394, 1977.
7. Ohnuma T, Roboz J, Shapiro ML, et al. Pharmacological and biochemical effects of pyrazofurin in humans. Cancer Res 37:2043–2049, 1977.
8. Douglass HO, Jr, Lavin PT, and Moertel CG. Nitrosoureas: useful agents for the treatment of advanced gastrointestinal cancer. Cancer Treat Rep 60:769–780, 1976.
9. Creagan ET, Rubin J, Moertel CG, et al. Phase II study of pyrazofurin in advanced colorectal carcinoma. Cancer Treat Rep 61:491–493, 1977.
10. Budman D, Currie V, and Wittes R. Phase II trial of pyrazofurin in malignant melanoma. Cancer Treat Rep 61:1733–1734, 1977.
11. Vogler WR, and Trulock PD. Phase I study of pyrazofurin in refractory acute myelogenous leukemia. Cancer Treat Rep 62:1569–1571, 1978.
12. Gralla RJ, Currie VE, Wittes RE, et al. Phase II evaluation of pyrazofurin in patients with carcinoma of the lung. Cancer Treat Rep 62:451–452, 1978.
13. Gralla RJ, Sordillo PP, and Magill GB. Phase II evaluation of pyrazofurin in patients with metastatic sarcoma. Cancer Treat Rep 62:1573–1574, 1978.
14. Cadman EC, Dix DE, and Handschumacher RE. Clinical, biological, and biochemical effects of pyrazofurin. Cancer Res 38:682–688, 1978.
15. Brenckman WD, Chu MY, and Fischer GA. Schedule dependence of the lethal effects of 6-azauridine and cytosine arabinoside on murine leukemia cells (L5178Y) in culture. Biochem Biophys Res Commun 52:1368–1373, 1973.
16. Brockman RW, Shaddix SC, and Rose LM. Biochemical aspects of chemotherapy of mouse colon carcinoma. Cancer 40:2681–2691, 1977.
17. DeConti RC, and Calabresi P. Treatment of polycythemia vera with azauridine and azaribine. Ann Intern Med 73:575–579, 1970.
18. Cornell RC, Milstein HG, Fox RM, et al. Anemia of azaribine in the treatment of psoriasis. Arch Dermatol 112:1717–1723, 1976.
19. McDonald CJ, and Calabresi P. Azaribine for mycosis fungoides. Arch Dermatol 103:158–167, 1971.

Phase II Trial of Piperazinedione in Malignant Melanoma: A Report by the Southeastern Cancer Study Group [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Forty-one evaluable patients with malignant melanoma resistant to other chemotherapeutic agents received 9 mg/m² of piperazinedione every 3 weeks. Two patients had partial responses and one additional patient had stable disease. One of the partial responses occurred in a patient with subcutaneous metastases and the other occurred in a patient with pulmonary and osseous metastases. Both antitumor responses occurred in the 17 patients with a performance status of ≥ 80%. The dose-limiting toxicity was myelosuppression; thrombocytopenia was more frequently observed than granulocytopenia.

[Cancer Treat Rep 63:1367–1369, 1979]

DTIC is the single most effective agent available for the treatment of malignant melanoma (1). Response rates as high as 25% have been reported (2). Somewhat better results have perhaps been achieved with DTIC in combination with other agents such as vincristine, procarbazine, BCNU, and bacillus Calmette-Guérin (3), or with cyclophosphamide (4). However, the majority of patients with malignant melanoma do not respond to chemotherapy, and the responses that do occur only last for periods of several months. There is therefore a need for the development of effective chemotherapy for patients who become drug-resistant.

In 1976, piperazinedione became available for phase II studies. It was demonstrated to be active against a large number of model tumors in mice, including Walker 256 carcinosarcoma and B16 melanoma (5). While the mechanism of action of the drug is unknown, it does have alkylating activity, is not cross-resistant with other alkylating agents, and produces a block in the G_2 phase of the cell cycle (6).

In 1975, phase I data indicated that two partial remissions (PRs) had been achieved in 16 patients with melanoma (7). The dose-limiting toxicity in that study was myelosuppression, and a dose of 15 mg/m² every 3 weeks was recommended in patients with adequate bone marrow reserve. Since unexpectedly severe myelosuppression was observed with the 15-mg/m² dose in several patients when piperazinedione was used alone or in combination with cyclophosphamide plus DTIC (8), we chose an initial dose of 9 mg/m². This report summarizes the experience of the Southeastern Cancer Study Group with piperazinedione in a phase II trial in patients with metastatic malignant melanoma between 1976 and 1978.

MATERIALS AND METHODS

Patients with biopsy-proven malignant melanoma which was incurable with standard techniques and was resistant to chemotherapy with cyclophosphamide plus DTIC, or DTIC alone, were eligible for this study. Patients were eligible regardless of performance status, site of metastatic disease, or liver or renal function. All patients were required to have at least one measurable lesion. Patients who had not recovered from the toxicity of prior therapy, who were pregnant, or who had a granulocyte count of $< 2000/mm^3$ or a platelet count of $< 100{,}000/mm^3$ were excluded from this study. Studies prior to therapy included history, physical examination, wbc and platelet counts, hemoglobin concentration, bone marrow biopsy, BUN, SGOT, LDH, alkaline phosphatase, bilirubin concentration, total protein and albumin concentration, and chest roentgenogram. Repeat blood cell counts were obtained 2 weeks after therapy and all studies were repeated prior to each course of therapy.

Piperazinedione was administered every 3 weeks at a dose of 9 mg/m² as an iv infusion over 10 minutes. If there was evidence of bone marrow involvement by the tumor, the initial dose was reduced to 7 mg/m². If the lowest

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granulocyte count was $> 2000/mm^3$ and the lowest platelet count was $> 125,000/mm^3$, the dose of piperazinedione in the subsequent course was increased by 3 mg/m^2. Therapy was always delayed until the granulocyte count had recovered to $> 2000/mm^3$ and the platelet count had recovered to $> 100,000/mm^3$. If the lowest granulocyte count was $< 750/mm^3$ or the lowest platelet count was $< 50,000/mm^3$, the dose of piperazinedione in the subsequent course was reduced by 50%.

A PR was defined as a $\geq$ 50% reduction in the sum of the products of the diameters of measurable lesions, with no progression of any single lesion. Progression of disease was defined as a > 25% increase in the sum of the products of the diameters of measurable lesions, or the appearance of new disease. Stable disease was less than a PR without progression of disease.

RESULTS

Fifty patients were entered in this study. Nine patients were inevaluable due to a lack of measurable lesions, a significant protocol deviation, or a refusal to receive further therapy. None of the patients refusing further therapy had an antitumor response. Refusal to receive further therapy was most often due to intolerance to moderate nausea and vomiting. Of the remaining 41 patients (table 1), two PRs were produced by piperazinedione. A PR lasting 3.5 months was obtained in a patient with subcutaneous metastases. The patient had previously received cyclophosphamide plus DTIC, and had a pretreatment performance status of 90%. No myelosuppression due to piperazinedione occurred in this patient. The other PR was observed in a patient with pulmonary and osseous metastases and lasted for 3 months. The patient had previously received cyclophosphamide plus DTIC and had a pretreatment performance status of 80%. Grade 2 platelet and grade 1 granulocyte toxicity were produced by the treatment (see below).

TABLE 1.—Summary of therapeutic results of piperazinedione in malignant melanoma

Patient characteristic	No. of patients treated	No. of PRs (duration)
Metastatic site		
Skin or lymph node only	10	1 (3.5 mos)
Any visceral organ	31	1 (3 mos)
Liver	9	0
Lung	19	1
Bone	15	1
Brain	3	0
Performance status (Karnofsky)*		
80%–100%	17	2
0–70%	21	0
Degree of myelosuppression†		
None or grade 1	34	1
Grade 2 or 3	7	1
All patients	41	2

*3 patients inevaluable for performance status.
†*See* text for definitions.

No responses were observed in patients with hepatic or brain metastases or in patients with a performance status of $\leq$ 70%. One additional patient had stable hepatic and osseous metastases for 3 months. This patient had previously received cyclophosphamide, DTIC, and methyl-CCNU and had a pretreatment performance status of 90%. The patient had grade 3 platelet and granulocyte toxicity.

Some degree of toxicity was achieved in 30 patients in this treatment program. Thirty-three patients had no granulocytopenia (lowest granulocyte count $> 1500/mm^3$); five had grade 1 toxicity (lowest granulocyte count between 750 and $1500/mm^3$); two had grade 2 toxicity (lowest granulocyte count $< 750/mm^3$); and one had grade 3 toxicity (sepsis).

Twenty-five patients had no significant thrombocytopenia (lowest platelet count $> 100,000/mm^3$); eight had grade 1 toxicity (lowest platelet count between 50,000 and $100,000/mm^3$); five had grade 2 toxicity (lowest platelet count $< 50,000/mm^3$); and two had grade 3 toxicity (gastrointestinal hemorrhage).

Of the three cases of grade 2 or 3 granulocytopenia, one occurred at a dose of 9 mg/m^2 and two occurred at doses of $\geq$ 12 mg/m^2. Of the eight cases of grade 2 or 3 thrombocytopenia, five occurred at a dose of 9 mg/m^2 and three occurred at doses of $\geq$ 12 mg/m^2.

Fourteen patients (34%) had a fall in hemoglobin concentration of > 1 g/dl; in seven patients the decrease was > 3 g/dl.

Nausea and vomiting were observed in 20 patients (severe in only two, however). One patient had cellulitis at the site of injection, followed by a sterile ulceration.

DISCUSSION

Only two PRs (5%) were observed in 41 patients with malignant melanoma resistant to other chemotherapeutic drugs. These negative results are in agreement with the results of Al-Sarraf et al (9) who observed no PRs in 28 patients at a dose of 12 mg/m^2 every 3 weeks. It is not surprising, therefore, that more toxicity was observed by those investigators; 72% of their patients had drug-induced toxicity, 30% had leukopenia, and 56% had thrombocytopenia, compared to 73%, 20%, and 37%, respectively, in our study. Despite the use of higher doses, Al-Sarraf et al failed to observe any significant antitumor responses.

Both of the antitumor responses in this report were observed in patients with a performance status of $\geq$ 80%. The additional patient with stable disease also had a per-

formance status of 90%. Only 17 patients had a performance status of ≥ 80%, and it may well be that piperazinedione, when used in patients with a high performance status, produces a marginal antitumor effect (two of 17 patients [12%]) of brief duration. Overall, piperazinedione is not a useful drug in the management of drug-resistant melanoma.

REFERENCES

1. Burke PJ, McCarthy WH, and Milton GW. Imidazole carboxamide therapy in advanced malignant melanoma. Cancer 27:744–750, 1971.
2. Nathanson L, Wolter J, Horton J, et al. Characteristics of prognosis and response to an imidazole carboxamide in malignant melanoma. Clin Pharmacol Ther 12:955–962, 1971.
3. Gutterman JU, Mavligit G, Gottlieb JA, et al. Chemoimmunotherapy of disseminated malignant melanoma with dimethyl triazeno imidazole carboxamide and bacillus Calmette-Guérin. N Engl J Med 291:592–597, 1974.
4. Presant CA, Bartolucci AA, Smalley RV, et al. Cyclophosphamide plus DTIC with or without *Corynebacterium parvum* in metastatic malignant melanoma. Cancer. In press, 1979.
5. Tarnowski GS, Schmid FA, Hutchison DJ, et al. Chemotherapeutic effects of compound 593A (NSC-135758) on mouse leukemias and some transplanted animal tumors. Cancer Chemother Rep 57:21–27, 1973.
6. Rao PN, and Mahagaokar S. The nature of piperazinedione-induced cytotoxicity in cultured cells. Proc Am Assoc Cancer Res and ASCO 16:74, 1975.
7. Gottlieb JA, Freireich EJ, Bodey GP, et al. Preliminary clinical evaluation of piperazinedione (P), a new crystalline antibiotic. Proc Am Assoc Cancer Res and ASCO 16:86, 1975.
8. Presant CA, and Klahr C. Severe myelosuppression from piperazinedione, (NSC-135758), cyclophosphamide plus dimethyl-triazeno-imidazole carboxamide (DTIC). Cancer 40:1386–1389, 1977.
9. Al-Sarraf M, Thigpen T, Groppe CW, et al. Piperazinedione in patients with advanced malignant melanoma: a Southwest Oncology Group study. Cancer Treat Rep 62:1101–1103, 1978.

Phase I Study of 5-Fluorodeoxyuridine Plus Cytosine Arabinoside Infusions in Patients With Solid Tumors [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

A phase I trial was conducted in 30 patients with solid tumors, using infusions of 5-fluorodeoxyuridine (5-FUdR) plus cytosine arabinoside (ara-C). Doses of 5-FUdR administered over 2 hours daily × 5 ranged from 0.02 to 1.0 mg/kg, and these doses immediately preceded a 1-hour infusion of ara-C. These schedules were selected because 5-FUdR pretreatment had been shown to sensitize L5178Y cells in culture to acute cell death by ara-C. Dose-limiting toxicity was myelosuppression. Based upon results in this study, the recommended dose of 5-FUdR to be used in phase II trials of this combination is 0.04–0.05 mg/kg (1.6–2.0 mg/m^2) given prior to ara-C at a dose of 100 mg/m^2. Antitumor responses were seen in patients with head and neck cancer, breast carcinoma, and carcinoid syndrome.

[Cancer Treat Rep 63:1371–1374, 1979]

The fluorinated pyrimidine, 5-fluorodeoxyuridine (5-FUdR), is an effective agent against several human solid tumors. It exerts its inhibitory action upon thymidylate synthetase, the enzyme necessary for the conversion of uridylate to thymidylate in de novo pyrimidine biosynthesis (1). Cytosine arabinoside (ara-C), on the other hand, has shown little antitumor activity against human solid tumors, even though it is an essential component of many successful combination chemotherapy programs for acute nonlymphocytic leukemias (2–5). Its mechanism of action is unknown, although several possibilities have been suggested. These include inhibition of the formation of deoxycytidine diphosphate from cytidine diphosphate, incorporation into DNA, inhibition of DNA polymerase, and incorporation into RNA (6–9). Experimentally, it has been demonstrated that pretreatment with relatively low doses of 5-FUdR can sensitize L5178Y cells in culture to acute cell death by ara-C. The mechanism by which this sensitization occurs is unknown. It may be related to 5-FUdR inhibition of ^{3}H-cytidine incorporation into DNA but not to the incorporation of ^{3}H-ara-C into DNA or RNA (10).

Based upon these observations and the known toxic effects of each agent when used alone, an early clinical trial was initiated in patients with advanced malignancies in order to determine an appropriate dosage schedule with tolerable toxicity. This report describes the results observed in the first 30 patients treated with this combination.

METHODS

Patient Selection

All of the patients evaluated in this study had progressive metastatic cancer which was felt to be incurable. Twelve of the 30 patients had received one or more types of conventional therapy prior to entry. Five had received radiation therapy only. Eight had been treated previously with chemotherapy and/or hormonal therapy. There had been no previous therapy within 4 weeks of entry. There were 17 males and 13 females. The median age was 56.7 years, with a range of 15–74 years. All of the patients had measurable or evaluable disease. The majority had normal pretreatment hepatic and renal functions, defined as SGOT ≤ 50 IU, LDH ≤ 200 units/100 ml, alkaline phosphatase ≤ 105 units/100 ml, total bilirubin ≤ 1.0 mg/100 ml, BUN ≤ 20 mg/100 ml, and/or serum creatinine ≤ 1.4 mg/100 ml. Pretreatment leukocyte, granulocyte, and platelet counts were normal in all patients. These biochemical and hematologic parameters were repeated weekly after treatment started. The types

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TABLE 1.—Patient characteristics

Tumor type	No. of patients	No. of responses			Survival (mos)
		PR	SD	Progression	
Breast	6	2	1	3	26+,8+,4+,1.4,1,0.5
Colon	7		3	4	6,4.7,4,3,2,1,1
Lung	3		2	1	5,3.7,1
Head and neck	3	1	2		4,3.7,1.2
Stomach	2			2	2,1
Miscellaneous (1 each)					
Carcinoid		1			12
Bladder			1		3
Prostate			1		4
Kidney				1	1
Lymphoepithelioma				1	1.5
Mesothelioma				1	0.5
Neuroblastoma				1	12.2
Rhabdomyosarcoma				1	0.5
Adenocarcinoma, unknown primary				1	3

of tumors in these patients are shown in table 1. Twenty-four of the patients had visceral metastases. Six had only osseous or soft tissue disease.

Dosage Schedules

Since the synergistic effect noted experimentally occurred only when 5-FUdR preceded a period of exposure to ara-C, a similar design was chosen for initiation of clinical studies. The relatively low doses of 5-FUdR ranged from 0.02 to 1.0 mg/kg (0.8–40 mg/m^2), and each was given as a 2-hour iv infusion. Ara-C at a dose of 100 mg/m^2 was given as a 1-hour infusion after the 5-FUdR. At the time this study was initiated, ara-C was commonly administered to leukemic patients at a dose of 100 mg/m^2 either by injection every 12 hours or by continuous infusion for 5 consecutive days. We selected a 1-hour infusion period for two reasons. The first reason is that ara-C is rapidly deaminated in man when given by bolus injection and synergy was noted only after a period of exposure to both 5-FUdR and ara-C. The second reason is that we did not want to reproduce in this group of patients with solid tumors the profound myelosuppression observed in leukemic patients treated with continuous 24-hour infusions of ara-C for 5 days. Both drugs in this study were repeated daily for a total of 5 days. Courses were repeated at monthly intervals until evidence of progressive disease occurred. The number of patients and trials studied at different 5-FUdR doses is shown in table 2. The initial starting dose of 5-FUdR was 1.0 mg/kg but this proved to be too myelotoxic, as did doses that were 50% and 40% of this starting level. There were patients at each of these doses who had leukocyte nadirs $< 1500/mm^3$. Thus, we lowered the daily dose of 5-FUdR to 0.02 mg/kg and escalated gradually from that level.

Response Criteria

A complete response was defined as the complete disappearance of all measurable disease. A partial response (PR) was defined as a reduction of measurable or evaluable metastases by at least 50% of the pretreatment level and/or recalcification of osseous metastases. Any patient not achieving either of the above responses but having a $< 25\%$ increase in the size of pretreatment parameters was classified as having stable disease (SD). All others had progressive disease.

RESULTS

Toxicity

The majority of patients had some nausea (21 of 30 patients) and vomiting (19 of 30 patients), beginning after several hours of treatment and usually not lasting for > 12 hours. This was easily controlled with antiemetics. One patient also developed chills during an infusion and another was found to have a generalized macular erythematous eruption after his third course of therapy. Dose-limiting toxicity was myelosuppression. The median leukocyte and platelet count depressions noted for each dose level evaluated are shown in table 2. The nadir platelet count occurred by Week 1 and the nadir leukocyte count occurred by Week 2 after starting treatment. No changes were noted in the levels of hemoglobin/

TABLE 2.—Dosage schedules of 5-FUdR given with ara-C*

5-FUdR dose (mg/kg/2 hrs × 5 days)	No. of trials/ No. of patients	Median nadir cell count × 10^3/mm^3 (range)	
		Wbcs	Platelets
0.02	2/2	4.5 (2.4–6.6)	160 (120–200)
0.025	3/2	2.5 (2.1–2.9)	350 (260–490)
0.04	27/13	3.0 (0.9–14.7)	157 (45–500)
0.05	10/10†	2.7 (2.1–16.3)	245 (50–440)
0.4	3/3	2.2 (1.3–2.7)	142 (27–185)
0.5	3/3†	2.7 (1.3–3.0)	163 (115–230)
1.0	1/1	1.4	65
Total	49/34‡		

*Ara-C was given as a constant dose of 100 mg/m^2 infused over 1 hr immediately following the 2-hr 5-FUdR infusion daily × 5.

†Includes 2 patients treated in earlier courses at other doses.

‡Includes 4 patients treated at several dosage levels.

hematocrit or in any biochemical parameters, except for those associated with disease progression in liver metastases. After dose escalations of 5-FUdR began at 0.02 mg/kg daily, there were only four instances of severe myelosuppression, defined as a wbc count of ≤ 2000/mm^3 and a platelet count of ≤ 50,000/mm^3. One patient reached nadir leukocyte counts of 900 and 1000/mm^3 in two courses and another patient's platelet count dropped to 45,000/mm^3 at a dose of 0.04 mg/kg. A third patient's platelet count fell to 50,000/mm^3 at a dose of 0.05 mg/kg. 5-FUdR given alone to two patients at a dose of 2 mg/kg over 2 hours daily for 5 days did not cause any depression of hematologic parameters.

Responses

The tumor types, number of patients treated, responses observed, and survival are shown in table 1. Overall, there were four PRs and ten SDs among the 30 patients treated. None of the responding patients had previously received 5-fluorouracil. Responses were evaluated based only upon the first dose level used in those patients who had treatment with different dose levels in subsequent courses. Two of six breast cancer patients had PRs and one other had SD lasting for 7, 3, and 3.5 months respectively. The most dramatic of these responses occurred in a 68-year-old woman who had extensive infiltration and induration of the skin of her left breast and entire left upper trunk anteriorly and posteriorly. She had an estimated 90% clearing of this disease after three courses at a 5-FUdR dose of 0.04 mg/kg/2 hours/day × 5. Thereafter, she was maintained on once weekly treatments at the same doses of 5-FUdR and ara-C, until she progressed 4 months later. Three of seven colon cancer patients had SD for 4.7, 4, and 3 months. Two of four lung cancer patients had SD for 3.7 and 3 months. The histologic types were squamous cell carcinoma and adenocarcinoma. Two other PRs were noted among the 30 patients treated. One of these was in a patient with metastatic carcinoma of the larynx, who had > 80% shrinkage of large left cervical nodes after completion of his first course of therapy. One month later, 10 hours after the first dose of his second course, the patient died suddenly from presumed erosion into his left carotid artery. The two other patients treated for squamous cell carcinoma of the head and neck had SD for 3 and 1.2 months respectively. One patient with carcinoid had a PR lasting for 12 months.

DISCUSSION

The purpose of this study was to determine a tolerable dose of 5-FUdR that could be administered preceding ara-C at a dose of 100 mg/m^2/day on each of 5 consecutive days at monthly intervals. The rationale for this sequence was based upon the demonstration that relatively low doses of 5-FUdR could sensitize L5178Y leukemic cells in culture to acute cell death caused by ara-C. This synergistic effect was not observed when both drugs were given simultaneously or when ara-C preceded 5-FUdR (10). From this study, we would recommend that the dose of 5-FUdR to be used in phase II trials of this combination be 0.04–0.05 mg/kg (1.6–2.0 mg/m^2). This dose results in acceptable myelosuppression when 5-FUdR is given for 5 consecutive days as a 2-hour infusion immediately preceding a 1-hour infusion of ara-C at a dose of 100 mg/m^2/day.

It was decided not to vary the dosage schedule of ara-C, in order to avoid the introduction of an additional variable in the escalation scheme. This decision was made because we anticipated some degree of myelosuppression simply from the dose of ara-C used, ie, 100 mg/m^2. The results presented in this study suggest that this was an appropriate decision. They do not, on the other hand,

preclude the development of other trials designed to evaluate the effects of altering either or both the dose and the duration of ara-C treatment used in combination with 5-FUdR. It might be anticipated that only limited increases in either the dose or duration of ara-C administration can be accomplished, however, in view of the degree of myelosuppression noted in this study with doses of 5-FUdR of > 0.05 mg/kg. It would seem reasonable, though, to evaluate these variables of ara-C treatment using doses of 5-FUdR that are ≤ 0.025 mg/kg, since severe myelosuppression was not observed at or below this level. It might also be possible to use an interval between courses of 3 weeks, instead of 4, since recovery from myelosuppression usually occurred within 1 week.

It is difficult to reach any conclusions regarding synergistic antitumor activity with this combination in man, since this study was primarily a phase I trial. All of the patients did have measurable or evaluable disease, however, and we did note antitumor activity in several patients who had previously received conventional therapy, but not with 5-FUdR, 5-fluorouracil, or ara-C. PRs were seen in two breast cancer patients, one head and neck cancer patient, and one patient with metastatic carcinoid syndrome. These were all of short duration, however.

It is possible that these results are due to single-agent activity of 5-FUdR and/or ara-C, but the doses of 5-FUdR (0.04–0.05 mg/kg) are only 4%–10% of the recommended 24-hour doses (0.5–1.0 mg/kg) used in continuous-infusion treatments, and ara-C has shown little activity against solid tumors (3).

The Eastern Cooperative Oncology Group has conducted a phase II trial of this combination in patients with advanced breast cancer, and these results are presently being analyzed. No phase II trials have been done in patients with head and neck cancer, but this is recommended for two reasons. First, this tumor is sensitive to other cycle-active agents, such as methotrexate and hydroxyurea, and second, we noted a dramatic regression in a patient with metastatic laryngeal carcinoma. The other types of tumors studied were too few to recommend use of this combination in phase II trials, but it probably should be considered for those diseases associated with a short doubling time or large growth fraction, where cell cycle-active agents or combinations have shown significant antitumor activity.

REFERENCES

1. Heidelberger C, Chaudhuri NK, Danneberg P, et al. Fluorinated pyrimidines, a new class of tumor-inhibitory compounds. Nature 179:663–666, 1957.
2. Talley RW. Clinical pharmacology and human antitumor activity of cytosine arabinoside. Cancer 20:809–816, 1967.
3. Wasserman TH, Comis RL, Goldsmith M, et al. Tabular analysis of the clinical chemotherapy of solid tumors. Cancer Chemother Rep Part 3, vol 6(2):399–419, 1975.
5. Crowther D, Powles RL, Bateman CJT, et al. Management of adult acute myelogenous leukemia. Br Med J 1:131–137, 1973.
6. Momparler RI, Chu MY, and Fischer GA. Studies on a new mechanism of resistance of L5178Y murine leukemia cells to cytosine arabinoside. Biochim Biophys Acta 161:481–493, 1968.
7. Chu MY, and Fischer GA. The incorporation of ^{3}H-cytosine arabinoside and its effect on murine leukemic cells (L5178Y). Biochem Pharmacol 17:753–767, 1968.
8. Furth JJ, and Cohen SS. Inhibition of mammalian DNA polymerase by the 5′-triphosphate of 1-β-D-arabinofuranosylcytosine and the 5′-triphosphate of 9-β-D-arabinofuranosyladenine. Cancer Res 27:1528–1533, 1967.
9. Chu MY. Incorporation of arabinosyl cytosine into 2-7S ribonucleic acid and cell death. Biochem Pharmacol 20:2057–2063, 1971.
10. Chu MY, Hoovis ML, and Fischer GA. Effects of 5-fluorodeoxyuridine on cell viability and uptake of deoxycytidine and [^{3}H]cytosine arabinoside in L5178Y cells. Biochem Pharmacol 25:355–357, 1976.

Hexamethylmelamine in Ovarian Cancer Resistant to Cyclophosphamide and Adriamycin [1]

Brief Reports and Preliminary Communications

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SUMMARY

Thirty-four patients resistant to cyclophosphamide and Adriamycin received hexamethylmelamine at one of two dose regimens: 6 mg/kg/day orally for 21 days every 4 weeks or 8 mg/kg/day orally for 21 days every 6 weeks. Only five patients responded. Tolerance to the drug was, however, satisfactory; nausea, pyrosis, and vomiting were the only frequent side effects. Myelosuppression occurred in only one case.

[Cancer Treat Rep 63:1375–1377, 1979]

Hexamethylmelamine (HMM) seems to be one of the most active drugs for the treatment of advanced epithelial ovarian cancer. Used as a single agent it has been reported to produce responses in about 40% of patients not previously treated with other antitumor agents (1).

An early study (2) in 15 patients with ovarian carcinoma reported encouraging results for the use of this compound in those who have become resistant to previous chemotherapy with alkylating agents; a response rate of 27% was reported. These results were confirmed in another study by Johnson et al who reported a response rate of 28% in 21 patients with refractory ovarian cancer (3).

The present study compared two dose regimens of HMM and attempted to determine its effectiveness in ovarian cancer patients not responding to, or relapsing after, cyclophosphamide (CYC) and Adriamycin (ADM) given in combination or sequentially.

PATIENTS AND METHODS

From November 1976 to September 1978, 34 patients with ovarian cancer (stage III) ranging in age from 27 to 71 years entered this study. They had already been treated with the usual surgical procedures and with the antitumor agents CYC and ADM; none of them had received radiation therapy. Their performance status was in the range of 60–100 and their life expectancy was at least 3 months.

The patients were stratified into one of two groups according to their previous chemotherapy. The first group (15 patients) had received CYC (100 mg/day orally continually) and ADM (50 mg/m^2 iv every 3 weeks) sequentially. ADM treatment had been started when the patients were evaluated as nonresponders (ie, they had either progressive or stable disease) after at least 3 months of CYC therapy, or after they had relapsed following an initial response to this drug.

A second group of 19 patients had received CYC (100 mg/day orally continually) in combination with ADM (50 mg/m^2 iv every 4 weeks). Nonresponders after at least 3 months, to ADM in the first group or to CYC plus ADM in the second group, became eligible for admission to this study.

After stratification the patients were randomly allocated to one of two HMM regimens: 6 mg/kg/day orally for 21 days every 4 weeks or 8 mg/kg/day orally for 21 days every 6 weeks. HMM was supplied by the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md) which also recommended the dosages. The median number of treatment cycles was three (range, one to five). Seven patients completed only one cycle because of disease progression.

Partial response was defined as a reduction in the sum of the tumor diameters by $> 50\%$ for at least 1 month. Minor response was defined as 25%–50% tumor reduction lasting at least 1 month, progression was defined as an increase in the sum of the tumor diameter by $> 25\%$ for at least 1 month, and no change was defined as a $< 25\%$ change.

Four patients were evaluated by laparoscopic examination because the tumor was not followable by physical pelvic examination (three had only diaphragmatic metastases and one had peritoneal metastasis).

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TABLE 1.—Responses to HMM (6- and 8-mg/kg dose regimens) in 34 ovarian cancer patients resistant to CYC and ADM

HMM (mg/kg)	Responses				
	Complete	Partial	Minor	No change	Progression
6	—	1	2	2	12
8	—	1	1	3	12
Totals (%)		2 (5.8)	3 (8.8)	5 (14.7)	24 (70.5)

RESULTS

Table 1 shows the responses seen in this study. Only five of 34 patients responded to HMM treatment: two had partial responses lasting 5 months and three had minor responses lasting 1.5, 2, and 2 months. Two had received the 8-mg/kg regimen and three had received the 6-mg/kg regimen. Four of these responders had previously received ADM and CYC in combination, and the fifth had received the same two drugs sequentially. Five of the 29 nonresponders had stable disease and 24 had progression. The overall median survival was 5 months (range, 1–15+) for nonresponders and 9 months (range, 4–12+) for responders. All but four patients had mild nausea and pyrosis. Thirteen had vomiting, with nine of these cases occurring during the higher dose regimen. No patient stopped treatment because of severe gastroenteric side effects. Drowsiness and paresthesia were observed in one patient, headache in three, conjunctivitis in two, and cystitis and pyrexia in one.

Median nadirs for wbc and platelet counts were 3900 and 180,000/μl respectively. In only one patient did the wbc and platelet counts fall to < 3000 and 100,000/μl and the lowest values were 2300 and 55,000/μl after three cycles of HMM therapy.

DISCUSSION

In this study we observed a poor response rate (14.7%) to HMM in ovarian cancer patients resistant to CYC and ADM, but good tolerance was noted in spite of previous chemotherapy. The very small number of responses makes it impossible to assess any difference between the two dose regimens. Also, in a previous pharmacokinetic study (4), we found such a high variability in oral absorption of HMM that any correlation between dose and effect appears unlikely. Johnson et al (3) reported the activity of HMM in ovarian carcinoma after alkylating agents had failed. The efficacy was low in their study also; they reported six objective responses among 21 patients, which is not statistically different from the five responses among 34 patients which we observed. The overall median survival was 8 months for nonresponders and 6 months for responders. The higher toxicity observed by Johnson et al is probably due to the different regimen employed: 8 mg/kg/day continually without intervals.

The apparently discouraging results with HMM in these refractory ovarian cancer patients are strikingly similar to those obtained with all other drugs that have been tested alone (5–15). In a recent report, Bruckner et al (16), using CYC, ADM, and *cis*-dichlorodiammineplatinum(II) in combination, found a response rate of 50%, much higher than the rates noted when the three drugs are used alone. Thus, perhaps polychemotherapy regimens should be tried as second-line chemotherapy to improve the poor results obtained until now.

In view of the good tolerance to the drug, work is now in progress to determine whether HMM in combination with other compounds can give better results in ovarian cancer patients resistant to conventional therapy.

REFERENCES

1. LEGHA SS, SLAVIK M, and CARTER SK. Hexamethylmelamine. An evaluation of its role in the therapy of cancer. Cancer 38:27–35, 1976.
2. BONOMI P, MORRIN B, WILBANKS G, ET AL. Phase II trial of hexamethylmelamine (HMM) in ovarian carcinoma after alkylating agent failure. Proc Am Assoc Cancer Res and ASCO 19:335, 1978.
3. JOHNSON BL, FISHER RI, BENDER RA, ET AL. Hexamethylmelamine in alkylating agent-resistant ovarian carcinoma. Cancer 42:2157–2161, 1978.
4. D'INCALCI M, BOLIS G, MANGIONI C, ET AL. Variable oral absorption of hexamethylmelamine in man. Cancer Treat Rep 62:2117–2119, 1978.
5. STANHOPE CR, SMITH JP, and RUTLEDGE F. Second trial drugs in ovarian cancer. Gynecol Oncol 5:52–58, 1977.
6. BARLOW JJ, and PIVER MS. Methotrexate (NSC-740) with citrovorum factor (NSC-3590) rescue, alone and in combination with cyclophosphamide (NSC-26271), in ovarian cancer. Cancer Treat Rep 60:527–533, 1976.
7. PIVER MS, BARLOW JJ, and CHUNG WS. High-dose cyclophosphamide (NSC-26271) for recurrent or progressive ovarian adenocarcinoma. Cancer Chemother Rep 59:1157–1158, 1975.
8. TWIGGS LB, and MORROW CP. Treatment of advanced ovarian carcinoma with high-dose cyclophosphamide after failure on melphalan. Cancer Treat Rep 61:1369–1371, 1977.
9. WILTSHAW E, and KRONER T. Phase II study of cis-dichlorodiammineplatinum(II) (NSC-119875) in advanced adenocarcinoma of the ovary. Cancer Treat Rep 60:55–60, 1976.
10. BOLIS G, D'INCALCI M, GRAMELLINI F, ET AL. Adriamycin

in ovarian cancer patients resistant to cyclophosphamide. Eur J Cancer 14:1401–1402, 1978.

11. HUBBARD SM, BARKES P, and YOUNG RC. Adriamycin therapy for advanced ovarian carcinoma recurrent after chemotherapy. Cancer Treat Rep 62:1375–1377, 1978.
12. OMURA G, DISAIA P, BLESSING J, ET AL. Chemotherapy for mustard-resistant ovarian adenocarcinoma: a randomized trial of CCNU and methyl-CCNU. Cancer Treat Rep 61:1533–1535, 1977.
13. EDMONSON JH, DECKER DG, MALKASIAN GD, ET AL. Brief communication: Phase II evaluation of cytembena (NSC-104801) in patients with advanced ovarian carcinoma resistant to alkylating agents. J Natl Cancer Inst 59:1619–1620, 1977.
14. MORASCA L, RECCHIA M, GRAMELLINI F, ET AL. Melphalan vs polymelphalanum in ovarian cancer patients resistant to cyclophosphamide: a tentative statistical approach to balance risks and benefits. Eur J Cancer 13:817–820, 1977.
15. EDMONSON JH, DECKER DG, MALKASIAN GD, ET AL. Phase II evaluation of VP-16-213 (NSC-141540) in patients with advanced ovarian carcinoma resistant to alkylating agents. Gynecol Oncol 6:7–9,1978.
16. BRUCKNER HW, RATNER LH, COHEN CJ, ET AL. Combination chemotherapy for ovarian carcinoma with cyclophosphamide, Adriamycin, and *cis*-dichlorodiammineplatinum(II) after failure of initial chemotherapy. Cancer Treat Rep 62:1021–1023, 1978.

Intermittent Pelvic Infusion With Vincristine, Bleomycin, and Mitomycin C for Advanced Recurrent Carcinoma of the Cervix [1]

Brief Reports and Preliminary Communications

Kenneth D. Swenerton,* John A. Evers, Gerald W. White, and David A. Boyes [2,3]

Palliation of progressive recurrent carcinoma of the uterine cervix presents difficult problems in the management of pelvic pain, fecal and urinary fistula, and ureteral obstruction leading to renal failure. A number of chemotherapy regimens have been explored in this disease and have utilized a variety of drugs both singly and in combination (1–10).

In two recently published studies using a combination of bleomycin and mitomycin C, in one case with vincristine (1,9), significant response rates have been recorded. We report our experience with this three-drug combination delivered as an arterial infusion.

MATERIALS AND METHODS

Between February 1976 and April 1978, a total of 20 patients with advancing recurrent carcinoma of the cervix were entered in this trial. The major criterion for admission to this study was biopsy-proven carcinoma of the uterine cervix persistent or recurrent in the pelvis following primary therapy. At entry in the trial, all patients had pelvic recurrence of disease, two had disease arising in the pelvis palpable in the abdomen, and none had disease elsewhere that could be detected by standard clinical, biochemical, or roentgenographic techniques. Physical examination to record extent of disease was performed by at least two observers. A chest roentgenogram was performed and blood obtained for hemogram, BUN, creatinine, LDH, SGOT, and alkaline phosphatase determinations. These hematologic and biochemical parameters were repeated every 2–3 weeks during the study. Chest roentgenograms were repeated prior to each treatment cycle.

Each of the 20 patients had received radical-dose radiotherapy consisting of a combination of intrauterine and intravaginal radium in addition to external cobalt irradiation. [4] In addition, two of these patients had undergone radical hysterectomy with pelvic node dissection, one patient had been given additional palliative radiotherapy, and three patients had received prior chemotherapy with the single agents melphalan, vincristine, and cyclophosphamide.

The patients were treated with vincristine, bleomycin, and mitomycin C delivered as an arterial infusion via an indwelling catheter introduced by the Seldinger technique through the femoral artery and sited in a position to deliver the greatest amount of drugs to the involved side of the pelvis by way of the appropriate common iliac artery. In cases of bilateral pelvic disease the catheter was sited at the bifurcation of the aorta. One of these 20 patients received two courses iv and a third course intra-arterially. The infusate consisted of heparinized normal saline (1 unit of heparin/cm^3) delivered at a constant rate of 60 cm^3/hour by a peristaltic pump.

Drugs were added to the infusate as follows:

1. Vincristine (2 mg) was given over 5 minutes with femoral tourniquets in position at Hour 0 and repeated at Hour 6. Tourniquets were inflated to a pressure sufficient to occlude femoral arterial flow and thereby restrict drug delivery as much as possible to the pelvic viscera.
2. Bleomycin (90 mg) was added to the infusate starting at Hour 12 and delivered continuously for 48 hours until Hour 60. This was accompanied by additional hydrocortisone (600–1500 mg), since local experience had led us to believe that the prophylactic use of corticosteroids could prevent certain unwanted side effects of bleomycin, particularly pyrexia and chills.
3. Mitomycin C (20 mg/m^2) was delivered as a continuous infusion from Hour 60 to Hour 66.

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[4]Combined point "A" doses were in the range of 5500–6000 rads and point "B" doses were in the range of 5100–6000 rads.

A maximum of three courses were to be given at 6-week intervals; however, the intervals varied, with 3-week intervals used in one case and a 13-week interval used in another. More frequent treatment was undertaken if toxicity from the previous course was inapparent. The number of courses was determined according to the anticipated dose-dependent pulmonary fibrosis from bleomycin. Nine patients received the planned three cycles of therapy, seven patients received two cycles, and four patients received one cycle.

Response was defined as follows: *complete response*—disappearance of all detectable disease for at least 1 month; *partial response*—incomplete disappearance of lesions, judged as > 50% of total initial tumor burden for at least 1 month; *progressive disease*—appearance of new lesions and any enlargement of existing lesions; and *stable disease*—any response not falling into any of the previous categories, including patients with a < 50% response.

RESULTS

Response

There were no complete responders. Three patients experienced a partial response at 9, 10, and 18 weeks after the start of treatment. Response was generally short-lived, relapse being detected at 27 and 38 weeks after entry into the study in two patients. One patient died from pulmonary fibrosis while in partial remission 13 weeks after entry in the study.

Nine patients were judged to have stable disease, of whom five had detectable but < 50% responses at 3, 4, 8, 12, and 13 weeks. This group showing minimal response demonstrated progressive disease at 12, 13, 14, 15, and 16 weeks after the start of treatment. Progression was evident in the remaining patients with stable disease at 11, 18, 23, and 36 weeks.

Seven patients had progressive disease and one patient who declined further therapy after one course could not be evaluated for response. Median survival was 30 weeks from the start of chemotherapy. Treatment was individualized following completion of this regimen, and no further comment on survival is of value except to say that two of the partial responders survived 93 and 113+ weeks.

The degree of histologic differentiation appeared to correlate with tumor response, in that five of seven patients with progressive disease had poorly differentiated tumors, while ten of 12 who had partial remission or stable disease had well- or moderately well-differentiated tumors.

Toxicity

Ten of 20 patients showed evidence of toxicity, three of these exhibiting symptoms in two separate organ systems.

Three of the nine patients receiving three cycles of treatment[5] developed symptoms or signs of pulmonary toxicity thought to be due to bleomycin; one died in respiratory failure having achieved partial remission of her cancer. An autopsy provided confirmatory evidence of bleomycin-induced pulmonary fibrosis. The patient who died was unique in that she had received bleomycin iv for two of the three courses administered.

The complications of arterial catheterization were one case of septicemia caused by *Staphylococcus aureus*, which was also associated with central retinal artery occlusion and blindness in one eye, and one case of an embolus in a single toe which quickly resolved with anticoagulation therapy.

There were three episodes of myelosuppression (wbc count, 1000–2000/mm^3), and in one of these cases prolonged neutropenia caused withdrawal of the patient from the protocol. None of these episodes was associated with infection. Thrombocytopenia and bleeding were not dose-limiting; in no instance was a platelet count of < 100,000/mm^3 recorded.

Vincristine was associated with two episodes of intestinal autonomic neuropathy when used at a dose of 4 mg/course in the initial 25 such courses given to the first 15 patients treated. Thereafter, the dose per cycle was reduced to 2 mg. Eighteen further courses were delivered at this reduced dose with one episode of neurotoxicity; all such episodes were self-limited.

In only one instance was the therapy associated with notable nausea and vomiting. Alopecia was observed in all patients.

DISCUSSION

The vascular supply to carcinoma of the cervix following surgery and radiation therapy may be expected to be compromised. In this study, drugs were delivered intra-arterially in an effort to achieve higher regional concentration and less systemic toxicity.

The rationale for introducing vincristine 12 and 6 hours before bleomycin was based on data showing that vincristine produces a marked increase in mitotic activity 6–12 hours after administration (11). Such a mitotic arrest could increase the efficacy of subsequently administered bleomycin, an agent which is cytotoxic in G_2 and M phases (12, 13). Bleomycin is rapidly taken up by squamous epithelium (14). The prolonged administration of bleomycin, and perhaps mitomycin C as well, to a tumor with a low growth fraction might logically be the most effective approach (10,15).

[5]The median dose of bleomycin administered to these three patients was 170 mg/m^2.

Unfortunately, as was the experience of Morrow et al (10), we have not demonstrated any practical advantage for this theoretic approach. Baker and his colleagues have recently published their experience with the same drugs given in three different schedules (1), one of which included a bleomycin infusion at comparable doses; they achieved responses in five of 18 patients with pelvic disease, but, as has been our experience, responses were short-lived. Neither the study by Baker et al nor the present study demonstrated response rates comparable to those achieved by Miyamoto et al (9) who found 12 complete responses and two partial responses among 15 Japanese patients treated with bleomycin and mitomycin C.

The ineffectiveness of this regimen is compounded by its unacceptable toxicity. The development of bleomycin-induced lung toxicity in three of our patients with death occurring in one patient suggests the need for extreme caution; measurements of diffusing capacity and vital capacity would appear to be mandatory before each course, even in the absence of symptoms or radiologic signs of pulmonary damage (16). The addition of corticosteroids, while effective in preventing pyrexia and chills during bleomycin infusion, clearly did not protect against pulmonary toxicity.

The results of our study suggest little benefit from using these drugs with an intra-arterial infusion technique. Further efforts to exploit intra-arterial therapy may be justified, particularly if more promising agents become apparent and known pharmacokinetic data are exploited.

REFERENCES

1. Baker LH, Opipari MI, Wilson H, et al. Mitomycin-C, vincristine, and bleomycin therapy for advanced cervical cancer. Obstet Gynecol 52:146–150, 1978.
2. Bond WH, Arthur K, Banks AJ, et al. Combination chemotherapy in the treatment of advanced squamous cell carcinoma of the cervix. Clin Oncol 2:173–178, 1976.
3. Malkasian GD, Jr, Decker DG, and Jorgensen EO. Chemotherapy of carcinoma of the cervix. Gynecol Oncol 5:109–120, 1977.
4. Conroy JF, Lewis GC, Brady LW, et al. Low dose bleomycin and methotrexate in cervical cancer. Cancer 37:660–664, 1976.
5. Hreshchyshyn MM. Vincristine treatment of patients with carcinoma of the uterine cervix. Proc Am Assoc Cancer Res 4:29, 1963.
6. Forney JP, Morrow CP, DiSaia PJ, et al. Seven-drug polychemotherapy in the treatment of advanced and recurrent squamous carcinoma of the female genital tract. Am J Obstet Gynecol 123:748–752, 1975.
7. Barlow JJ, Piver MS, Chuang JT, et al. Adriamycin and bleomycin, alone and in combination, in gynecologic cancers. Cancer 32:735–743, 1973.
8. Greenberg BR, Kardinal CG, Pajak TF, et al. Adriamycin versus Adriamycin and bleomycin in advanced epidermoid carcinoma of the cervix. Cancer Treat Rep 61:1383–1384, 1977.
9. Miyamoto T, Takabe Y, Watanabe M, et al. Effectiveness of a sequential combination of bleomycin and mitomycin-C on an advanced cervical cancer. Cancer 41:403–414, 1978.
10. Morrow CP, DiSaia PJ, Mangan CF, et al. Continuous pelvic arterial infusion with bleomycin for squamous carcinoma of the cervix recurrent after irradiation therapy. Cancer Treat Rep 61:1403–1405, 1977.
11. Frei E III, Whang J, Scoggins RB, et al. The stathmokinetic effect of vincristine. Cancer Res 24:1918–1925, 1964.
12. Barranco SC, and Humphrey RM. The effects of bleomycin on survival and cell progression in Chinese hamster cells in vitro. Cancer Res 31:1218–1223,1971.
13. Livingston RB, Bodey GP, Gottlieb JA, et al. Kinetic scheduling of vincristine (NSC-67574) and bleomycin (NSC-125066) in patients with lung cancer and other malignant tumors. Cancer Chemother Rep 57:219–224, 1973.
14. Umezawa H. Bleomycin: discovery, chemistry and action. *In* GANN Monograph on Cancer Research (Fundamental and Clinical Study of Bleomycin) (Carter SE, et al, eds). Baltimore, Md, University Park Press, 1976, vol 19, pp 3–36.
15. Buckles RG. New horizons in drug delivery. Ca 28:343, 1978.
16. Boushy SF, and North LB. Pulmonary function in infiltrative lung disease. Chest 64:448–453, 1973.

Phase II Study Demonstrating Failure of Both a Five-Drug Continuous-Therapy Regimen and a Two-Drug Pulse-Therapy Regimen in the Treatment of Metastatic Neuroblastoma: Southwest Oncology Group Study 822 [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Twenty-six children > 1 year of age with previously untreated stage IV neuroblastoma were randomized to receive either a five-drug regimen (prednisone, cyclophosphamide, actinomycin D, vincristine, and daunorubicin) or a two-drug regimen (cyclophosphamide and vincristine). Complete response rates were 6% and 9% for the five-drug and the two-drug regimens respectively. Partial response rates were 13% and 27% for the five-drug and the two-drug regimens respectively. The mean duration of the seven responses was 9 months, and all 26 patients had died within 2 years. Neither regimen was effective for long-term disease control in these children with neuroblastoma.

[Cancer Treat Rep 63:1383–1384, 1979]

A number of drugs have been shown to have activity in neuroblastoma. From 1971 to 1973 the Pediatric Division of the Southwest Oncology Group hoped to find that a multiple-drug combination would prove more effective than a two-drug combination in the management of advanced neuroblastoma. Our rationale was an extrapolation of the success encountered with multiple-drug combinations in the management of Hodgkin's disease and acute leukemia.

MATERIALS AND METHODS

Patients

Patients > 1 year of age having stage IV neuroblastoma with a measurable tumor and without previous chemotherapy were eligible for the study. There was no stratification with respect to presence or absence of bone metastases between the two regimens. After written informed consent had been given by the parents or guardians, patients were randomized between the two treatment arms. Following randomization, the ages of the patients in the two treatment regimens were, fortuitously, closely matched.

Drug Programs

The five-drug regimen consisted of prednisone, 60 mg/m^2 given orally daily (maximum dose, 60 mg/day) from Day 1 to Day 28, with tapering of the drug in 1 week; cyclophosphamide, 70 mg/m^2 given orally daily from Day 1 to Day 28; actinomycin D, 450 μg/m^2 given iv daily (maximum dose, 500 μg) for the first 5 days; vincristine, 1.5 mg/m^2 given iv weekly × 4; and daunorubicin, 25 mg/m^2 given iv weekly × 4 (maximum cumulative dose, 450 mg) starting on Day 28. Except for prednisone, all drugs were repeated at 8-week intervals. The two-drug regimen consisted of vincristine, 1.5 mg/m^2 given iv weekly × 8 (maximum dose, 2.0 mg), and then every 3 weeks × 4; and cyclophosphamide, 1000 mg/m^2 given every 2 weeks for a total of four doses, and then every 4 weeks.

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The therapy was to be discontinued 12 months after the last tumor disappearance, or at the time of recurrence or disease-progression. Patients who were treated for < 8 weeks were considered not evaluable unless treatment was discontinued because of progressive disease. Physical examination and a cbc were required weekly, with evaluation of specific tumors to be reported monthly. A bone marrow aspiration and roentgenographic skeletal survey were evaluated every 12 weeks.

The chemotherapy responses were rated in a standard way: complete remission (disappearance of all tumors); partial remission (decrease in the product of cross-sectional diameters of measurable tumors by ≥ 50%); no response (no decrease in the size of tumors); and progressive disease (increase in the size of tumors).

RESULTS

Twenty-six patients were admitted to the study and 13 of the 15 patients in the five-drug regimen were evaluable. All 11 patients in the two-drug regimen were evaluable. One of the 13 evaluable patients receiving the five-drug regimen entered complete remission and relapsed after 352 days in the study. Two additional patients had a partial remission and relapsed after 168 and 184 days in the study respectively. One of the 11 evaluable patients receiving the two-drug regimen entered complete remission and relapsed after 658 days in the study. Three additional patients had a partial remission while receiving this regimen and relapsed after 158, 198, and 226 days in the study. Twelve of the 13 patients receiving the five-drug regimen had leukopenia, infection, or mouth sores requiring a 30%–40% reduction of drug dose. Only two of the 11 patients receiving the two-drug regimen required drug dose modification for significant leukopenia or intolerable vomiting. Mean survival from the start of treatment was 186 days for the five-drug regimen and 259 days for the two-drug regimen. Although symptom-free survival time could not be accurately ascertained for either treatment group, the degree of toxicity encountered suggests strongly that those patients receiving the two-drug regimen encountered far fewer side effects and presumably had more symptom-free days of survival.

DISCUSSION

Aggressive combination chemotherapy for stage IV neuroblastoma was not demonstrably superior to a more conventional two-drug regimen. The apparent superiority of the two-drug regimen is most likely explained by our failure to stratify patients entering the study according to the extent of tumor involvement. Ten of the 13 evaluable patients receiving the five-drug regimen had bony involvement while only six patients receiving the two-drug regimen had bony lesions. Future randomized studies must include stratification of patients to ensure comparable extent of disease in patients in each treatment arm. Substratification should at least include known prognostic factors such as age, presence of bony lesions, histology, location of the primary tumor, and perhaps the peripheral lymphocyte count (1). For purposes of accurately comparing different therapeutic approaches, it seems that these additional criteria regarding the prognostic features of stage IV disease must be applied uniformly within the various oncology groups and major institutions reporting results of neuroblastoma treatment.

REFERENCE

1. Grosfeld JL, Schatzlein M, Ballantine TVN, et al. Metastatic neuroblastoma: factors influencing survival. J Pediatr Surg 13:59–65, 1978.

Use of Vinblastine in the Terminal Phase of Chronic Myelocytic Leukemia [1]

Brief Reports and Preliminary Communications

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SUMMARY

Vinblastine (6 mg/m^2) was given to 15 patients in the terminal phase of Ph^1 chromosome-positive chronic myelocytic leukemia and to one patient with chronic myelomonocytic leukemia. In each of these patients, there was prompt reduction in leukocyte counts (median, 80% decrease). Effects on platelet counts and hematocrit levels were inconsistent. Nine patients had decreases in the percentage of circulating blast cells. Reduction in splenomegaly and relief of bone pain were recorded in patients with these manifestations. Serious leukopenia was induced in only one patient, and was of brief duration. Additional doses of vinblastine at intervals of ≥ 3 weeks were given to eight patients who had shown improvement in the differential cell count, as an adjunct to maintenance schedules of combination chemotherapy. Good results were obtained initially, but the quality and duration of responses decreased after two to six injections.

[Cancer Treat Rep 63:1385–1387, 1979]

In the last 2 decades, vinblastine (VBL) has had wide use in the treatment of malignant diseases. Its principal side effect is a consistent and prompt depression of the granulocyte count. Despite this effect, there has been little investigation of its use in myeloid leukemia (1,2), while another vinca alkaloid, vincristine (VCR), with less effect on the leukocyte count and with more serious toxic potential, has had more extensive trials.

We felt that the dependable leukopenic effect of VBL, with platelet-sparing and freedom from serious side effects, warranted a trial of the agent among patients with chronic myelocytic leukemia (CML) who were not responding to more conventional chemotherapy.

METHODS AND MATERIAL

Fifteen patients with Ph^1 chromosome-positive CML and one patient with chronic myelomonocytic leukemia were treated. All patients met the criteria of Karanas and Silver (3) for the terminal phase of CML.

There were 11 males and five females who were 17–79 years of age. Median duration of disease prior to VBL therapy was 21 months (range, 1–82). Median duration of the terminal phase was 4 months (range, 1–18). Ten patients had had prior splenectomy as part of the management of the terminal phase of CML (4). Terminal deoxynucleotidyl transferase (TdT) was determined in four patients and increased enzyme activity was found in three.

VBL was used as the initial agent in four patients. These patients were started on combinations of trimethylcolchicinic acid and 6-mercaptopurine (5) 7–10 days after their first dose of VBL. In the other 12 patients, VBL was added to ongoing schedules of combination chemotherapy (principally, with colchicine derivatives and purine analogs) when lack of control became evident, as manifested by increasing leukocytosis, increase in the percentage of blast cells, or progressive splenic enlargement. A dose of 6 mg/m^2 was selected for this study. Patients who were receiving other antileukemic agents at the time of VBL administration continued receiving the same drugs, at the same or lower doses, until after the effects of VBL were evaluated.

RESULTS

Reduction in the leukocyte counts (55%–95%; median, 80%) was seen in all patients (fig 1). Onset of effect was within 48 hours and the nadir was reached 3–9 days after the VBL injection (median, 6 days). Effects on the platelet counts were variable, with no significant change for the group as a whole. Decrease in the percentage of circulating blast cells, usually accompanied by an increase in the percent of mature neutrophils, was recorded in nine patients.

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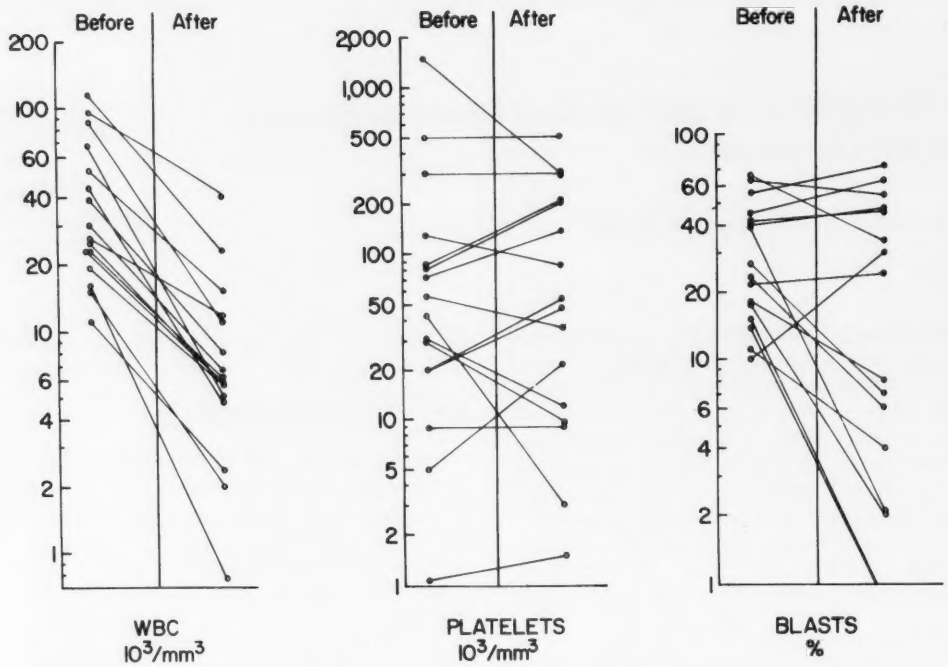

FIGURE 1.—Effects of the first dose of VBL on the leukocyte and platelet counts and the percentage of circulating blast cells among 16 patients.

Relief of bone pain (four patients) and decreases in splenomegaly (six patients) were observed in all patients in whom those parameters could be evaluated. Lymphadenopathy and pulmonary infiltrates in the patient with myelomonocytic leukemia resolved completely.

No difference was noted in the acute effects of VBL between the four patients without other therapy and the 12 patients receiving concurrent two-drug combinations.

Eight patients whose differential cell counts improved received additional doses of VBL at intervals of 3–12 weeks (median, 4) while receiving maintenance chemotherapy with combinations of two oral drugs. After several doses, the decreases in leukocyte counts became smaller (eg, 30%–40% versus 80%) and the durations of response became progressively shorter. Loss of the favorable effect on the differential cell count sometimes occurred as early as after the second injection.

Responses to VBL did not correlate with TdT levels. One patient with high and one with low TdT activity had clinical and hematologic benefit. Both received additional doses of VBL and remained in satisfactory control for 6 and 8 months respectively after entry in this study. Two other patients with high TdT levels had only minor and transient benefit. Both also failed to respond to VCR and prednisone.

Toxicity

Leukopenia of < 1000 cells/mm^3 was seen in one patient. Two other patients had decreases in their wbc counts to < 3000/mm^3. Recovery occurred within 1 week in all three patients. Four patients had decreases in their hematocrit levels immediately after VBL administration and three patients had thrombocytopenia. However, the relationship between VBL administration and these abnormalities was difficult to evaluate because of the unstable status of these patients. Mild to moderate alopecia was seen among some patients who were also receiving colchicine derivatives. No gastrointestinal side effects were seen. No patient had symptoms of neurotoxicity, although there was unequivocal decrease in tendon reflexes in three patients.

DISCUSSION

VBL regularly produced a prompt and relatively predictable decrease in leukocyte counts in this population of poor-risk patients, most of whom had already shown resistance to other chemotherapeutic agents. Relief of bone pain and decrease in splenomegaly paralleled the effect on leukocytosis. Half of these patients had im-

provement in the differential cell counts. This has more long-term significance in the terminal phase of CML than reduction in leukocyte count, and is more difficult to obtain. Serious toxicity was rare and of transient duration.

Other treatment routines frequently used to obtain rapid control of increasing leukocytosis among similar patients, such as high iv doses of cyclophosphamide, continuous infusions of cytosine arabinoside, and large and frequent doses of hydroxyurea, commonly produce distressing gastrointestinal side effects, and the degree and duration of wbc count depression induced by such routines may be difficult to predict. Our experience suggests that VBL deserves consideration as an additional agent for rapid reduction of the leukocyte count. Its advantages include relatively predictable effects on wbc count, improvement in the differential cell count in 50% of patients, minimal side effects, and, possibly, less danger of serious leukopenia.

In contrast to experience with VCR (6), TdT activity was not a prerequisite for VBL effect. Of particular interest is the fact that prior therapy with demecolcine or trimethylcolchicinic acid, drugs with similar effects in metaphase, did not prejudice response to VBL.

The beneficial effects of VBL, especially on the differential cell count, did not extend beyond 6–8 months. While this is probably no worse than the duration of response to VCR (6) and a number of other agents with some effectiveness in terminal CML, it is not enough to produce a major change in survival of such patients.

Mathé et al (7) have recently reported a 50% response rate in blastic CML to treatment with vindesine, a derivative of VBL. A comparison of the two agents would be of interest, and it would be desirable to determine whether there is cross-resistance between them.

REFERENCES

1. Carbone PP, Tjio TH, Whang J, et al. The effect of treatment in patients with chronic myelogenous leukemia. Ann Intern Med 59:622, 1963.
2. Hill JM, and Loeb E. Treatment of leukemia, lymphoma, and other malignant neoplasms with vinblastine. Cancer Chemother Rep 15:41–61, 1961.
3. Karanas A, and Silver RT. Characteristics of the terminal phase of chronic granulocytic leukemia. Blood 32:445, 1968.
4. Gomez GA, Sokal JE, Mittelman A, et al. Splenectomy for palliation of chronic myelocytic leukemia. Am J Med 61:14, 1976.
5. Gomez GA, Sokal JE, and Aungst CW. Chemotherapy of the terminal phase of chronic myelocytic leukemia with combination of colchicine derivatives and purine analogs. Leukemia Res 2:141, 1978.
6. Marks SM, Baltimore D, and McCaffrey R. Terminal transferase as a predictor of initial responsiveness to vincristine and prednisone in blastic chronic myelogenous leukemia. N Engl J Med 298:812, 1978.
7. Mathé G, Misset JL, De Vassal F, et al. Phase II clinical trial with vindesine for remission induction in acute leukemia, blastic crisis of chronic myeloid leukemia, lymphosarcoma, and Hodgkin's disease: absence of cross-resistance with vincristine. Cancer Treat Rep 62:805–809, 1978.

Evidence for Toxicologic Activity of Ftorafur Independent of Conversion to 5-Fluorouracil [1,2,3]

Brief Reports and Preliminary Communications

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Ftorafur is the N_1-tetrahydrofuran-2-yl derivative of 5-fluorouracil (5-FU). Ftorafur yields 5-FU in vivo (1,2), and the clinical effectiveness and toxicity of ftorafur have been attributed to 5-FU (3,4). Early clinical and pharmacologic studies of ftorafur have been interesting because ftorafur crosses the blood-brain barrier (1,2,5), and daily iv bolus administration of ftorafur without myelosuppression is better tolerated clinically than some 5-day 5-FU treatment regimens (3–5).

These and subsequent studies (2,5–8) have raised questions of whether the biologic activity of ftorafur can be accounted for solely by production of 5-FU. Some have suggested (5–7) that ftorafur toxicity may result from metabolic intermediates that may make little or no contribution to the therapeutic activity of ftorafur. In the course of our studies of the mouse model as a predictor of the clinical toxicity of anticancer drugs, we evaluated the toxicity of ftorafur and found gastrointestinal changes qualitatively similar to those produced by 5-FU (9). The onset of these changes was much earlier than after 5-FU however, and we report here the principal results of these studies.

MATERIALS AND METHODS

Ftorafur was obtained from the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). The drug was suspended in aqueous NaCl (0.9 g/100 ml) that contained hydroxypropyl cellulose (0.3 g/100 ml; Carter-Glogau Laboratories, Melrose Park, Ill). Ftorafur concentrations were prepared so that 0.1 ml/10 g of body weight provided the doses desired for mice.

Duplicate experiments were carried out. Young adult male B6D2F$_1$ mice (Simonsen Laboratories, Gilroy, Calif) weighing 24–27 g each were used. The mice were housed in individual stainless steel cages with hardwood bedding (Betta-Chip, Northeastern Products Corp, Warrensburg, NY) and received Wayne Lab-Blox F6 (Allied Mills, Inc, Chicago, Ill) and tap water ad libitum. In each experiment, the mice were randomly distributed into five treatment groups of 70 mice each.

Each mouse was treated with a single ip dose of 340, 510, 680, or 850 mg/kg of ftorafur which represents the estimated 0.6, 0.9, 1.2, and 1.5 LD10. Doses were administered on the basis of individual body weights obtained prior to treatment. In each experiment one group of 70 mice was treated with the hydroxypropyl cellulose diluent. On posttreatment Days 1, 3, 6, 10, 14, and 21 in each experiment, ten mice from each treatment group were exsanguinated by cardiac puncture under chloroform anesthesia. Five mice from each group provided individual blood samples for hematologic evaluation and samples of liver, kidney, spleen, stomach, duodenum, jejunum, ileum, and colon for histopathologic evaluation. The remaining five mice provided individual blood samples for determination of 16-test clinical chemistry profiles. Ten mice from each treatment group were observed for 30 posttreatment days, and deaths were recorded.

The methods used for comprehensive hematologic and clinical chemistry evaluations in mice have been described (10). Tissue samples for histopathologic evaluation were collected and preserved in buffered formalin (10 g of formaldehyde/100 ml of phosphate buffer, pH 7). Tissue samples were embedded in Paraplast, sectioned at 4–6 μm, and stained with Harris' hematoxylin and eosin (11).

RESULTS

Probit analysis (12) of lethality data from these and previous experiments indicated that the lethal doses (95% confidence limits) for single-dose ip administration of ftorafur to male B6D2F$_1$ mice are: LD10, 583 mg/kg (507–632); LD50, 771 mg/kg (727–821); and LD90, 1019 mg/kg (933–1189). These results are in reasonable agreement with those of Johnson et al (6).

The sublethal doses of ftorafur used produced reticulocytopenia (data not shown) with a nadir on Day 3 (day

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[5]We wish to acknowledge the technical assistance of Anne M. Cusic, Ruth G. Crosby, Vergia Askew, and the staff of the Preclinical Pharmacology and Toxicology Division.

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TABLE 1.—Epithelial hypertrophy, hyperplasia, and macronucleosis in the colons of mice treated with ftorafur

Dose		Experiment	Day after treatment			
mg/kg	mg/m²	No.	1	3	6	10
0	0	1	0/5*	0/5	—	0/5
		2	0/5	0/5	0/5	0/5
340	1020	1	5/5(1.0)†	5/5(1.2)	5/5(1.0)	0/5
		2	3/5(0.6)	5/5(1.0)	2/5(0.4)	0/5
510	1530	1	5/5(1.4)	4/5(0.8)	4/5(0.8)	3/5(0.6)
		2	3/5(0.6)	5/5(1.4)	2/5(0.4)	0/5
680	2040	1	5/5(2.0)	5/5(2.0)	5/5(1.2)	0/4
		2	1/5(0.2)	5/5(1.0)	3/5(0.8)	0/5
850	2550	1	2/5(0.4)	3/5(0.6)	5/5(2.0)	—
		2	1/5(0.2)	3/3(1.4)	2/4(0.5)	0/4

*Numerator = No. of affected mice; denominator = No. of mice examined.
†No. in parentheses = mean degree of severity (0 = no lesion, 1 = minimal, 2 = moderate, and 3 = severe).

of treatment = Day 0) and granulocytopenia with a nadir on Day 6. Both cell populations returned to normal by Day 14. These changes were generally dose-dependent, and the cell counts were below the 2.5th percentile reference value established in a large control population (10). Clinical chemistry evaluations did not suggest any specific target organ toxicity. Reductions of plasma glucose, BUN, protein, and electrolyte concentrations on Days 3–6 reflected posttreatment morbidity and weight loss. Recovery was complete on about Day 14. These observations generally confirm and extend those of Johnson et al (6). Thrombocytopenia was not observed in the present studies.

Histopathologic evaluation of tissue samples revealed a mild but persistent epithelial hyperplasia of the stomach, small intestine, and colon (table 1) consistent with early regeneration of damaged mucosae. The changes were evident 24 hours after treatment, and recovery was not complete until about Day 10. The effects of ftorafur on gastrointestinal epithelia were qualitatively similar to those observed in mice treated with 5-FU (9). Worthy of emphasis in table 1 is the evidence of ftorafur toxicity on Day 1, which is much earlier than changes observed after 5-FU treatment (9).

DISCUSSION

Although ftorafur is converted to 5-FU in several animal species (1,6–8,13) and in man (2,5), the gastrointestinal toxicity of ftorafur we observed in mice was not consistent with a hypothesis that the biologic activity of ftorafur may be attributable solely to 5-FU. Some of these studies (2,7,8) have revealed ftorafur metabolites that may be intermediates in the conversion of ftorafur to 5-FU. No definite role has yet been ascribed to any of these metabolites as contributors to the pharmacologic or toxicologic activity of ftorafur. In light of what we know about the gastrointestinal toxicity of 5-FU in mice (9), microscopic changes in the gastrointestinal epithelia observed in the present studies would not be expected within the first 24 hours after treatment with ftorafur unless the toxicity were attributable to the unchanged drug or a metabolite other than 5-FU. At equitoxic doses, gastrointestinal changes induced in mice by 5-FU were not detectable until at least 48 hours after treatment. Additional work, including synthesis and evaluation of ftorafur metabolites, will be required to determine the mechanism of ftorafur toxicity.

REFERENCES

1. COHEN AM. The disposition of ftorafur in rats after intravenous administration. Drug Metab Dispos 3:303–308, 1975.
2. BENVENUTO JA, LU K, HALL SW, ET AL. Disposition and metabolism of 1-(tetrahydro-2-furanyl)-5-fluorouracil (ftorafur) in humans. Cancer Res 38:3867–3870, 1978.
3. SMART CR, TOWNSEND LB, RUSHO WJ, ET AL. Phase I study of ftorafur, an analog of 5-fluorouracil. Cancer 36:103–106, 1975.
4. VALDIVIESO M, BODEY GP, GOTTLIEB JA, ET AL. Clinical evaluation of ftorafur (pyrimidine-deoxyribose N_1-2′-furanidyl-5-fluorouracil). Cancer Res 36:1821–1824, 1976.
5. HALL SW, VALDIVIESO M, and BENJAMIN RS. Intermittent high single-dose ftorafur: phase I clinical trial with a pharmacologic-toxicity correlation. Cancer Treat Rep 61:1495–1498, 1977.
6. JOHNSON RK, GARIBJANIAN BT, HOUCHENS DP, ET AL. Comparison of 5-fluorouracil and ftorafur. I. Quantitative and qualitative differences in toxicity to mice. Cancer Treat Rep 60:1335–1345, 1976.
7. WU AT, AU JL, and SADEE W. Hydroxylated metabolites of *R,S*-1-(tetrahydro-2-furanyl)-5-fluorouracil (ftorafur) in rats and rabbits. Cancer Res 38:210–214, 1978.
8. SADEE W, AU JL, WU AT, ET AL. Metabolic activation of 1-(tetrahydrofuran-2-yl)-5-fluorouracil (ftorafur). Proc Am Assoc Cancer Res and ASCO 19:92, 1978.
9. HARRISON SD, JR, DENINE EP, and PECKHAM JC. Qualita-

tive and quantitative toxicity of single and sequential sublethal doses of 5-fluorouracil in BDF_1 mice. Cancer Treat Rep 62:533–545, 1978.
10. Harrison SD, Jr, Burdeshaw JA, Crosby RG, et al. Hematology and clinical chemistry reference values for C57BL/6 × $DBA/2F_1$ mice. Cancer Res 38:2636–2639, 1978.
11. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed (Luna LG, ed). New York, NY, McGraw-Hill Book Co, 1968, pp 32–39.
12. Finney DJ. Probit Analysis, 3rd ed. Cambridge, England, Cambridge University Press, 1971.
13. Freudenthal RI, and Emmerling DC. The metabolism of ftorafur in the beagle dog and rhesus monkey. Xenobiotica 7:757–764, 1977.

Ophthalmopathy After Neck Irradiation Therapy for Hodgkin's Disease [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

Ophthalmopathy associated with autoimmune thyroid disease (Graves' ophthalmopathy) may occur years after irradiation of the neck for nonthyroid malignant disease. Two patients developed this complication 2 and 18 years after irradiation treatment of Hodgkin's disease: one with hypothyroidism associated with Hashimoto's thyroiditis and the other with hyperthyroidism. Careful long-term observation of thyroid function following neck irradiation is recommended in view of this unusual complication and the frequent occurrence of hypothyroidism.

[Cancer Treat Rep 63:1393–1395, 1979]

The etiology of the ophthalmopathy associated with Graves' disease and less commonly, Hashimoto's thyroiditis, remains unknown, although an autoimmune process may be involved. The severity of the eye findings is often unrelated to the degree of hyperthyroidism. Ophthalmopathy may occur before, during, or after hyperthyroidism or when the patient is hypothyroid; it may be unilateral. The occurrence of ophthalmopathy after neck irradiation for malignant disease has recently been described by Wasnich et al (1) and Wenzel et al (2). The present report describes the occurrence of severe ophthalmopathy in association with diffuse toxic goiter in one patient and with hypothyroidism and Hashimoto's thyroiditis in another, years after irradiation to the neck for Hodgkin's disease.

CASE REPORTS

Patient 1

A 26-year-old female nurse developed right anterior cervical node enlargement in 1972. A biopsy and subsequent studies, including a lymphangiogram, confirmed the diagnosis of stage IIA Hodgkin's disease, with restriction of the disease to the mediastinum and right neck. Exploratory laparotomy was not carried out. She was treated with X-ray therapy through a mantle field (4000 rads). Her family history was negative for thyroid disease.

The patient first noted exophthalmos 2 years later, but did not consult a physician until January 1975. At that time, her serum T_4 concentration was elevated to 15.9 μg/dl while she was taking oral contraceptives. Thyroid-radioactive iodine uptake was elevated and a triiodothyronine suppression test revealed a lack of thyroid suppression. She was then treated with 200 mg of propylthiouracil (PTU) daily. She was first seen at the University of Massachusetts Medical Center in October 1975 complaining of severe diplopia and nervousness. Physical examination revealed a pulse of 110 beats/minute and a twice normal, diffusely enlarged thyroid. She had Plummer's nails. Bilateral proptosis, lid lag, and mild conjunctival injection were present. The right eye deviated medially and the lateral gaze was markedly impaired. Exophthalmometer readings were 21 mm bilaterally. The serum T_4 was 17.4 μg/dl (normal = 4–11.5 μg/dl), the serum rT_3U was 40.7% (normal = 41%–55%), and the free thyroxine index was 14.7 (normal = 3.4–13.3). Antithyroglobulin antibodies were positive at a titer of 1:25 and antimicrosomal antibodies were strongly positive at a titer of 1:1600. At that time the patient was taking 200 mg of PTU daily and oral contraceptives. She was treated with 60 mg of prednisone daily and 1 week later extra-ocular movements were normal and symptoms of diplopia had disappeared. Steroids were gradually decreased and discontinued 4 months later. PTU was discontinued in December 1976. She is now euthyroid, but the exophthalmos still persists. Serum thyroid stimulating hormone-displacing immunoglobulin (TDI) was absent in 1978.[4] The patient has no clinical evidence of Hodgkin's disease.

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Patient 2

A 50-year-old woman developed bilateral anterior cervical and left axillary node enlargement in 1958. A diagnosis of Hodgkin's lymphoma was confirmed by biopsy. A lymphangiogram and exploratory laparotomy were not carried out. The patient was treated with mechlorethamine and radiation therapy of 2800 rads to her right cervical region in 1958 and an additional 2600 rads to her left cervical region in 1961. Hypothyroidism and prominent eyes developed in 1971 and she was treated with 1 grain of dessicated thyroid daily. Her family history was negative for thyroid disease.

This patient was first seen at the University of Massachusetts Medical Center in April 1976 with bilateral proptosis and diplopia. Physical examination revealed dry skin, clubbing of nails, delayed relaxation of the deep tendon reflexes, and a two-times enlarged, firm thyroid. Bilateral lid lag and exophthalmos and impaired lateral and superior lateral gaze were present. Exophthalmometer readings were 22 mm (right eye) and 24 mm (left eye). Serum T_4 concentration was almost undetectable at 0.4 μg/dl, serum thyroid stimulating hormone concentration was strikingly elevated to > 100 μU/ml, and serum antimicrosomal and antithyroglobulin antibodies were positive at titers of 1:25,600, strongly suggesting the diagnosis of hypothyroidism secondary to Hashimoto's thyroiditis. Serum TDI[5] was not present. Therapy with 40 mg of prednisone and 0.15 mg of sodium-L-thyroxine daily was begun. One week later, ophthalmoplegia had definitely improved and over the following 14 months the prednisone was gradually reduced and discontinued. At present she is euthyroid and is receiving 0.15 mg of sodium-L-thyroxine daily; the only eye findings are persistent bilateral exophthalmos. There is no evidence of Hodgkin's disease.

DISCUSSION

A retrospective review of 70 patients with Graves' ophthalmopathy by Wasnich et al (1) revealed seven patients who had previously received irradiation therapy to the head and neck. Three patients had received radiation treatment for lymphoma, two for breast cancer, one for laryngeal carcinoma, and one for lymphoepithelioma. Four of these patients were hyperthyroid, two were euthyroid, and one was hypothyroid at the time they presented with ophthalmopathy. All patients were considered to have had Graves' disease. There was no previous history of thyroid disease. Ophthalmopathy appeared over a period of 1½–7 years following radiation therapy. The patient reported by Wenzel et al (2) was a 60-year-old woman who developed ophthalmopathy and hyperthyroidism 10 years following external radiation for breast cancer. However, the patient had been receiving lithium for 3 years, a drug reported to be associated with exophthalmos (3). More recently, the occurrence of thyrotoxic Graves' disease but only mild exophthalmos was reported in one patient 4 years after irradiation to the neck for Hodgkin's disease (4).

Wasnich et al (1) and Wenzel et al (2) have postulated that radiation damage to the thyroid might induce release of thyroid antigens which could stimulate formation of the thyroid stimulating immunoglobulins, the TDIs or the antithyroid auto-antibodies commonly associated with Graves' disease, or the antithyroid antibodies almost always present in patients with Hashimoto's thyroiditis. It has been postulated, but not as yet proven, that the pathogenesis of Graves' ophthalmopathy is due to an autoimmune process in the retro-orbital tissue and eye muscles. Delayed hypersensitivity to retrobulbar and thyroid tissue has been demonstrated in patients with Graves' exophthalmos (5). Konishi et al (6) observed in vitro binding of thyroglobulin to extra-ocular muscle membranes, with secondary binding of thyroglobulin-antithyroglobulin immune complexes, and Kriss (7) demonstrated a common pathway of lymph drainage from the thyroid gland and the orbit in a patient with untreated Graves' disease and acute exudative ophthalmopathy. Thus, antigen release from the thyroid following irradiation could directly reach the orbit and later result in immune complex precipitation and subsequent ophthalmopathy. High titers of antithyroid antibodies were present in the two patients in the present report, both of whom had severe ophthalmopathy. TDI was not detected in the sera from these patients but would not be expected to be present in patient 2 with Hashimoto's thyroiditis and hypothyroidism and serum was only available for the TDI assay in the patient with hyperthyroidism (patient 1) approximately 2 years after the hyperthyroidism was in remission.

Wasnich et al (1) considered the possibility that the occurrence of Graves' disease was coincidental in their patients who were destined to develop this disease regardless of the associated neck irradiation. It has been reported that approximately 50% of white patients with Graves' disease have the HLA-B8 histocompatibility antigen, whereas this antigen is found in only 22% of controls (8). However, the HLA-B8 antigen was not present in the eight patients with postradiation ophthalmopathy (1,2). It seems probable, therefore, that the ophthalmopathy following head and neck irradiation is secondary to the previous therapy and not merely coincidental. HLA typing was not carried out in our two patients.

Antithyroid antibodies associated with myxedema have been detected in the sera of five patients (9) and in two of three patients (10) following irradiation to the neck for nonthyroid neoplasms. Antithyroid antibodies were also found in four of 18 euthyroid patients following ^{131}I therapy for angina pectoris (11) and in eight of 31 patients

[5] *See* footnote 4.

following ^{131}I therapy for angina, compression symptoms secondary to goiter, and ablation of thyroid remnants following surgery for thyroid carcinoma (12). Although Hashimoto's thyroiditis might have been present prior to the radiation therapy, these observations suggest that radiation damage to the thyroid could induce autoimmune thyroiditis and subsequent hypothyroidism.

Adler et al (10) have recently reviewed the literature concerning the occurrence of hypothyroidism after radiation therapy to the head and neck for nonthyroid lymphoma and other malignancies. The incidence of clinical hypothyroidism or an elevated serum thyroid stimulating hormone occurring 2 months to 16 years after radiation therapy ranged from 4% to 44% and was especially common in patients who received a large iodine load from lymphangiography (13) or who had a partial thyroidectomy (14). Iodide-induced hypothyroidism is common in euthyroid patients previously treated with ^{131}I or surgery for thyrotoxic Graves' disease and in patients with Hashimoto's thyroiditis (15). High-dose external radiation to the neck also appears to increase the sensitivity of the thyroid to the blocking effects of iodide on thyroid hormone synthesis. In the present report, the patient with Hashimoto's thyroiditis and hypothyroidism (No. 2) had not had a lymphangiogram. The patient who developed hyperthyroidism (No. 1) had had a lymphangiogram 2 years earlier but the presence of an elevated thyroid ^{131}I uptake strongly suggested that iodide had not induced the hyperthyroidism (Jod-Basedow).

Regardless of the etiology, ophthalmopathy following external neck irradiation for nonthyroid neoplasms appears to be a distinct entity. Careful long-term followup of thyroid function in patients receiving high-dose irradiation to the neck for nonthyroid malignant disease seems warranted in view of the relatively high incidence of hypothyroidism and the less common complication of hyperthyroid Graves' disease and/or Graves' ophthalmopathy.

REFERENCES

1. Wasnich RD, Grumet FC, Payne RO, et al. Graves' ophthalmopathy following external neck irradiation for nonthyroidal neoplastic disease. J Clin Endocrinol Metab 37:703–713, 1973.
2. Wenzel KW, Schleusener H, and Weise W. Durch externe schilddrüsenbestrahlung ausgelöste Hyperthyreose und endokrine Ophthalmpathie. Hinweis auf immunologische Pathogenese. Dtsch Med Wochenschr 99:2036–2039, 1974.
3. Segal LS, Rosenblatt S, and Eliasoph I. Endocrine exophthalmos during lithium therapy of manic-depressive disease. N Engl J Med 289:136–138, 1973.
4. Pilepich MV, Jackson I, Munzenrider JE, et al. Graves' disease following irradiation for Hodgkin's disease. JAMA 240:1381–1382, 1978.
5. Mahieu P, and Winand R. Demonstration of delayed hypersensitivity to retrobulbar and thyroid tissues in human exophthalmos. J Clin Endocrinol Metab 34:1090–1092, 1972.
6. Konishi J, Herman MM, and Kriss JP. Binding of thyroglobulin and thyroglobulin-antithyroglobulin immune complex to extraocular muscle membrane. Endocrinology 95:434–446, 1974.
7. Kriss JP. Radioisotopic thyroidolymphography in patients with Graves' disease. J Clin Endocrinol Metab 31:315–324, 1970.
8. Grumet FC, Payne RO, Konishi J, et al. HL-A antigens as markers for disease susceptibility and autoimmunity in Graves' disease. J Clin Endocrinol Metab 39:1115–1119, 1974.
9. Markson JL, and Flatman GE. Myxoedema after x-ray therapy to the neck. Br Med J 1:1228–1230, 1965.
10. Adler RA, Corrigan DP, and Wartofsky L. Hypothyroidism after x irradiation to the neck: three case reports and a brief review of the literature. John Hopkins Med J 138:180–184, 1976.
11. Burke G. Dissociation between thyroid injury and formation of long-acting thyroid stimulator in thyrotoxicosis. Ann Intern Med 67:349–355, 1967.
12. Einhorn J, Fegraevs A, and Jonsson J. Thyroid antibodies in euthyroid subjects after iodine-131 therapy. Radiat Res 28:296–301, 1966.
13. Glatstein E, McHardy-Young S, Brast N, et al. Alterations in serum thyrotropin (TSH) and thyroid function following radiotherapy in patients with malignant lymphoma. J Clin Endocrinol Metab 32:833–841, 1971.
14. Shafer RB, Nutall FQ, Pollak K, et al. Thyroid function after radiation and surgery for head and neck cancer. Arch Intern Med 135:843–846, 1975.
15. Vagenakis AG, and Braverman LE. Adverse effects of iodides on thyroid function. Med Clin North Am 59:1075–1088, 1975.

ICRF-159 (Razoxane) in the Treatment of Pediatric Solid Tumors: A Southwest Oncology Group Study[1,2]

Brief Reports and Preliminary Communications

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SUMMARY

ICRF-159 is active in several animal tumor model systems and human adult malignancies. In this phase II study, ICRF-159 was given on a weekly schedule, 3000 mg/m²/day, orally in three divided doses at 6-hour intervals to 78 children with a variety of malignant neoplasms. Fifty-three patients were evaluable for tumor response. Toxicity was primarily hematopoietic and gastrointestinal. There were no responses in any of the eight patients with osteogenic sarcoma, four with lymphoma, five with Ewing's sarcoma, ten with neuroblastoma, or six with rhabdomyosarcoma. There was a transient partial response in one of four children with Wilms' tumor. Further trials with this drug using this schedule are not indicated for the common childhood solid tumors.

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ICRF-159 was developed in England and was first selected for clinical studies because of its demonstrated antitumor activities in the murine L1210 leukemia and murine Lewis lung carcinoma model systems (1). Objective responses have been reported in patients with colorectal carcinoma, Kaposi's sarcoma, acute lymphocytic leukemia, and non-Hodgkin's lymphoma (2). In addition to its direct antineoplastic effect, it has shown several interesting effects in animal studies: it inhibits metastatic development in mice with Lewis lung carcinoma (3), it is a radiosensitizer in mice with sarcoma S180 (4), and it has a cardiotoxicity-sparing action on the isolated dog heart when given with Adriamycin or daunorubicin (5).

This is a report of a phase II study of this drug used in a weekly dose regimen in children with a variety of solid tumors. The results of similar studies on pediatric leukemic patients will be reported separately.

METHODS

Seventy-eight patients with a variety of advanced solid malignant tumors who were < 19 years of age at the time of diagnosis were entered in this study. All had previously received chemotherapy, were resistant to all standard forms of chemotherapy, and had a life expectancy of > 6 weeks. Each patient had a measurable lesion with which to assess the efficacy of ICRF-159, and all had recovered from previous significant drug toxicity. Twenty-five of these patients were not evaluable for tumor response because of early death, but they were included in the toxicity assessment.

The patients were to receive a 6-week course of ICRF-159, 3 g/m²/week given orally on 1 day in three divided doses at 6-hour intervals. The drug was supplied by the National Cancer Institute (Bethesda, Md) as 500- and 50-mg tablets. Six weeks of therapy, or only 3 weeks in the face of progressive tumor growth, were necessary for a patient to be considered evaluable for antitumor response. The definition of a partial response was a reduction by at least 50% in the product of the diameters of a measurable lesion; a complete remission required the disappearance of all measurable lesions.

RESULTS

The numbers of patients with each tumor type evaluable for antitumor response are shown in table 1. The only response in a common childhood tumor was a transient partial reduction in the abdominal girth of a child with a protruberant abdomen due to widespread intra-abdominal Wilms' tumor which lasted only 8 weeks. A

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TABLE 1.—Tumor types

Tumor	No. of patients
Neuroblastoma	10
Osteosarcoma	8
Rhabdomyosarcoma	6
Ewing's sarcoma	5
Wilms' tumor	4
Lymphoma (3 Hodgkin's, 1 non-Hodgkin's)	4
Neurofibrosarcoma	2
Testicular carcinoma	2
Sacrococcygeal teratoma	2
Hepatocarcinoma	2
Hemangioendothelioma	1
Fibrosarcoma	1
Retinoblastoma	1
Undifferentiated carcinoma	1
Ovarian teratocarcinoma	1
Brainstem glioma	1
Astrocytoma	1
Schwannoma	1
Total	53

TABLE 2.—Toxicity in 78 patients

Type of toxicity	No. of patients
Leukopenia (cells × 10^9/liter)	
<4.0 >3.0	19
≤3.0 >2.0	17
≤2.0 >1.0	31
≤1.0	11
Thrombocytopenia (cells × 10^9/liter)	
≤100.0 >50.0	18
≤50.0 >25.0	4
≤25.0	7
Nausea and vomiting	
Mild	9
Moderate	4
Severe	1
Diarrhea	2

child with hepatocarcinoma died as a result of his disease during the fourth week of treatment, at which time the palpable tumor had decreased in size by 50%.

The toxicity of the drug schedule was quite acceptable, with significant hematopoietic depression being the main side effect (table 2). Although the degree of leukopenia was life-threatening in 11 patients ($\leq 1.0 \times 10^9$ cells/liter) and the thrombocytopenia was life-threatening in seven patients ($\leq 25.0 \times 10^9$ cells/liter), none of the early deaths were related to this myelosuppression nor were there any cases of sepsis or hemorrhage. Since almost all patients exhibited some degree of leukopenia, further drug dose escalation using this schedule would produce unacceptable myelosuppression. Gastrointestinal toxicity, as shown by nausea, vomiting, or diarrhea, was both infrequent and mild.

COMMENT

It is apparent that ICRF-159 given as a weekly "pulse" is ineffective in the commonly occurring pediatric tumors, the only response in this latter group of tumors being a brief and partial response in a child with Wilms' tumor. We would conclude that further such trials with this schedule are not indicated. Perhaps this drug will find a role in pediatric oncology if further studies confirm the report that it is an effective radiosensitizer for metastatic soft tissue sarcoma (6).

REFERENCES

1. CREIGHTON AM, HELLMANN K, and WHITECROSS S. Antitumor activity in a series of bis-diketopiperazines. Nature 222:384–385, 1969.
2. BELLET RE, ROZENCWEIG M, VON HOFF DD, ET AL. ICRF 159: current status and clinical prospects. Eur J Cancer 13:1293–1298, 1977.
3. HELLMANN K, and BURRAGE K. Control of malignant metastases by ICRF-159. Nature 224:273–275, 1969.
4. HELLMANN K, and MURKIN GF. Synergism of ICRF 159 and radiotherapy in treatment of experimental tumors. Cancer 34:1033–1039, 1974.
5. HERMAN EH, MHATRE RM, LEE IP, ET AL. Prevention of the cardiotoxic effects of Adriamycin and daunomycin in the isolated dog heart. Proc Soc Exp Biol Med 140:234–239, 1972.
6. HELLMANN K, RYALL RHD, MACDONALD E, ET AL. Comparison of radiotherapy with and without razoxane (ICRF-159) in the treatment of soft tissue sarcomas. Cancer 41:100–107, 1978.

Phase II Trial of Vindesine in Patients With Germ-Cell Tumors [1,2]

Brief Reports and Preliminary Communications

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Vindesine, a vinblastine derivative, began clinical trial in 1975 (1). It has an antitumor spectrum in animals similar to that of vincristine, but with less neurotoxicity (2,3). Phase I trials of vindesine at Memorial Sloan-Kettering Cancer Center explored both a weekly and a daily × 5 dose schedule. A dose of 1.3 mg/m²/day × 5–7 days every 3 weeks was well-tolerated. There were two measurable remissions in six heavily pretreated patients with testicular cancer (4). A phase II trial of vindesine in patients with germ-cell tumors who had failed to respond to conventional chemotherapy was begun in January 1977 and this is a report of the results.

MATERIALS AND METHODS

Vindesine was supplied in 10-mg vials by Eli Lilly and Co, Indianapolis, Ind. It was mixed with an accompanying diluent to a final drug concentration of 1 mg/ml and was administered slowly through a free-flowing iv infusion, since extravasation causes local cellulitis. Twenty patients with germ-cell tumors were treated on a daily schedule. The initial course of vindesine was 1.3 mg/m²/day × 5–7 consecutive days. Patients received a course of therapy every 3 weeks if measurable disease remained stable or improved, with dose adjustments being made to maintain acceptable toxicity. The overall dose range was 1.0–1.5 mg/m²/day × 5–8 days. Pretreatment evaluation included complete medical history and physical examination, Karnofsky performance status (PS), chest roentgenogram, alpha-fetoprotein (AFP), serum beta subunit of human chorionic gonadotropin (HCG), lactic dehydrogenase, cbc, and platelet count. Other studies were done when indicated. Cbcs and platelet counts were repeated daily during therapy and at least weekly thereafter. Chest roentgenograms and other blood tests were repeated every 2–4 weeks.

Partial remission (PR) is defined as a > 50% reduction in the sum of the products of the perpendicular diameters of all measurable disease, lasting for at least 4 weeks and accompanied by improvement or stabilization of PS. An adequate trial is defined as completion of at least one course of vindesine with subsequent leukopenia and a survival of at least 4 weeks from the initiation of therapy.

Patient Characteristics

Twenty male patients were entered in this study from January 1977 to November 1978. Sixteen patients had testicular germ-cell tumors, two had retroperitoneal germ-cell tumors, and two had mediastinal germ-cell tumors. The median age at study entry was 25 years (range, 16–44 years) and the median PS was 60 (range, 40–100). The patients had received an average of 17 months of chemotherapy (range, 7–30 months) before entering the phase II vindesine trial. After failing to respond to previous therapy, they remained untreated for an average of 7 weeks (range, 1–34 weeks) before receiving their first dose of vindesine. Thirteen of the 20 patients (65%) had had prior radiation therapy. Nineteen patients have died of their disease. One patient is still alive with disease. The median time from original diagnosis to death was 24 months (range, 10–42 months). The median time from the beginning of vindesine treatment to death was 11 weeks (range, 1–54 weeks).

RESULTS

All of the 20 patients received at least one full course of vindesine. One patient was inevaluable because he died of a pulmonary embolism on the fifth day of his only course of vindesine. The remaining 19 patients had an adequate trial, and all had evaluable disease. Elevated AFP and/or HCG values were seen in 18 of 19 patients

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(six, only AFP elevated; two, only HCG elevated; and ten, both values elevated). Among the 19 patients, nine had one evaluable course of vindesine, five had two courses, two had three courses, two had five courses, and one had six courses.

Three of the 19 patients had PRs. One PR was in a 29-year-old male with testicular seminoma metastatic to bone and soft tissues. He had a disappearance of soft tissue disease lasting for > 12 weeks with an increase in his PS from 60 to 80, though bone roentgenograms showed no improvement. He remains in PR after three courses of vindesine. Another PR was in an 18-year-old male with testicular embryonal carcinoma metastatic to the lungs and retroperitoneum. His pulmonary disease improved for 7 weeks and then stabilized for 6 more weeks before clear progression was seen. His AFP level returned to normal for 2 months, along with a > 50% decrease in HCG from pretreatment values. His PS remained at 90 throughout six courses of vindesine. The third PR was in a 35-year-old male with embryonal carcinoma of the mediastinum metastatic to the lungs and pleura. His pulmonary disease improved markedly over 4 weeks and remained stable for an additional 4 weeks before clear progression was seen. His AFP value was < 50% of the pretreatment value for 4 weeks. His PS increased from 50 to 90 during his five courses of vindesine.

Toxicity

The dose-limiting toxic effect was leukopenia, defined as a wbc count of < 3500/mm^3; it was seen in at least one course in all of the 19 patients. The mean wbc count nadir was 1850/mm^3 (range, 500–3500/mm^3); it occurred an average of 12 days (range, 4–17 days) after vindesine treatment began. A wbc count nadir of < 1000/mm^3 was seen after eight vindesine courses, and occurred in five patients. Wbc recovery was noted by Day 19 (range, Days 11–27). The mean platelet count nadir was 163,000/mm^3 (range, 37,000–295,000/mm^3); it was noted 8 days (range, 1–22 days) after a course of vindesine treatment began. As noted in previous studies (4), the platelet count nadir was seen more quickly than the wbc count nadir and was not as marked. Thrombocytosis was not seen in this study.

Vindesine was well-tolerated. Six patients had no complaints attributable to vindesine. The most commonly noted problems were paresthesias (nine patients), constipation (six), fever (six), and mucositis (six), though these toxic effects never prevented the completion of therapy. An opportunistic infection was not proven in the two patients whose fevers were associated with neutropenia and mucositis, though their fevers resolved with antibiotics and wbc count recovery. Vomiting and moderately severe asthenia occurred in a few patients. Since most patients had lost their hair with previous chemotherapy, the incidence of alopecia could not be determined. There were no deaths attributable to vindesine.

DISCUSSION

Using vinblastine as a single agent, Samuels and Howe (5) observed four complete remissions and seven PRs in 21 patients with testicular cancer. However, only two of their patients had previously received any chemotherapy, and neither had had vincristine. Vindesine, a vinblastine derivative, should also be effective in germ-cell tumors. Smith and his colleagues in England (6) recently reported that vindesine caused one PR and one minor remission in five patients with testicular teratoma. In our trial, vindesine caused clinically beneficial PRs in three of 19 evaluable patients (16%), all of whom were heavily pretreated with combination chemotherapy.

Since previous chemotherapy can affect the response to subsequent drugs, the relationship of the response to vindesine to prior vinblastine therapy was examined. All of the 19 patients had received a median total dose of 106.8 mg (range, 42.4–210 mg) of vinblastine delivered over an average of 17 doses (range, six to 37 doses). The three patients who responded to vindesine were in a subgroup of eight patients who had received a total dose of < 80 mg of vinblastine. The durations of their previous chemotherapy were 14, 11, and 10 months, respectively; all of these durations were less than the treatment group median of 14 months. One patient, the man with seminoma, achieved a PR with vindesine after having become resistant to vinblastine as a single agent. He had received 63.5 mg of vinblastine in six doses over 10 weeks, with a minor remission lasting 8 weeks. Three weeks without chemotherapy separated the last dose of vinblastine from the first dose of vindesine.

The overall response of three of 19 patients, with these three responses being among eight patients who were in the less heavily pretreated subgroup, demonstrates that vindesine is active on a daily schedule in germ-cell tumors, though it is not clearly better than vinblastine. Since all but one patient had received prior vinblastine in combination with other drugs, it is impossible to determine if the patients were refractory to further vinblastine as a single agent. However, these results suggest that vindesine has activity similar to vinblastine in germ-cell tumors and that heavy prior vinblastine therapy markedly decreases the likelihood of subsequent response to vindesine. Any conclusions regarding the replacement of vinblastine with vindesine in the primary therapy for germ-cell tumors would require a randomized trial of the two drugs.

REFERENCES

1. DYKE RW, and NELSON RL. Phase I anti-cancer agents. Vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135–142, 1977.
2. SWEENEY MJ, BODER GB, CULLINAN GJ, ET AL. Antitumor activity of deacetyl vinblastine amide sulfate (vindesine) in rodents and mitotic accumulation studies in culture. Cancer Res 38:2886–2891, 1978.
3. TODD GC, GIBSON WR, and MORTON DM. Toxicology of vindesine (desacetyl vinblastine amide) in mice, rats, and dogs. J Toxicol Environ Health 1:843–850, 1976.
4. CURRIE VE, WONG PP, KRAKOFF IH, ET AL. Phase I trial of vindesine in patients with advanced cancer. Cancer Treat Rep 62:1333–1336, 1978.
5. SAMUELS ML, and HOWE CD. Vinblastine in the management of testicular cancer. Cancer 25:1009–1017, 1970.
6. SMITH IE, HEDLEY DW, POWLES TJ, ET AL. Vindesine: a phase II study in the treatment of breast carcinoma, malignant melanoma, and other tumors. Cancer Treat Rep 62:1427–1433, 1978.

Phase II Trial of Pyrazofurin in Patients With Multiple Myeloma Refractory to Standard Cytotoxic Therapy [1,2,3]

Brief Reports and Preliminary Communications

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Pyrazofurin (PZF), a C-nucleoside with activity against transplanted tumors in rodents (1,2), exerts its cytotoxic activity by inhibition of orotidylate decarboxylase (2–4). It has produced stomatitis, dermatitis, and orotic acidemia in phase I trials (3–6), and it lacked therapeutic activity against melanoma (7), lung (8) and colorectal (9) cancer, and metastatic sarcoma (10) in phase II evaluation. This is a report of a phase II trial of PZF in 14 heavily pretreated patients with plasma cell myeloma.

MATERIALS AND METHODS

Patient characteristics are shown in table 1. Prior to entry in this trial these patients had received therapy with combinations of alkylating agents, BCNU, prednisone, and vincristine for a median duration of 38 months (range, 5–59 months). All patients had measurable disease parameters, including a protein spike on serum protein electrophoresis and/or free light chains in the urine.

Pretreatment evaluation included complete medical history and physical examination, cbcs, SMA-12, creatinine, protein electrophoresis and immunoelectrophoresis of serum and urine, complete urinalysis, creatinine clearance, chest roentgenogram, electrocardiogram, and bone marrow aspiration for routine smears and labeling index.

Followup evaluation included weekly serum protein electrophoresis, 24-hour urines for protein electrophoresis, and immunoelectrophoresis. Other abnormal parameters were followed at weekly or biweekly intervals, except for bone roentgenograms which were followed at 4-month intervals. The response criteria of the Myeloma Task Force were utilized in this study (11).

PZF was administered iv at a weekly dose of 3 mg/kg, with attenuation if renal failure or marked mucositis occurred. All patients received the drug on an outpatient basis. There was no concurrent antineoplastic treatment.

RESULTS

PZF produced one partial remission (PR) of 5 months' duration, a minor regression that continues at 18 months, and a stabilization of previously progressing disease in three additional patients for 7+, 8+, and 11 months (table 1). The PR was characterized by a > 90% reduction in a massive pelvic plasmacytoma and disappearance of the monoclonal "spike" from the serum electrophoretic

TABLE 1.—Patient characteristics and results of PZF therapy in 14 evaluable patients with plasma cell myeloma

Characteristic	No. of patients
Age in yrs	
Median: 60	
Range: 49–69	
Performance status (ref 13)	
Median: 50%	
Range: 40%–70%	
Disease stage (ref 14)	
II	1
III	13
Toxic effects	
Hypercalcemia	3
Hyperviscosity	3
Hemoglobin < 10 g	8
Leukocyte count < 4000/mm^3	2
Platelet count < 140,000/mm^3	2
Prior chemotherapy	14
Response	
Partial	1 (5)*
Minor	1 (18+)
Stable	3 (7+, 8+, 11)
Progression	9

*Nos. in parentheses = response duration in mos.

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pattern. The patient achieving minor regression has had a sustained 20% reduction in his protein spike on serum protein electrophoresis. Before therapy, he required monthly plasmapheresis to prevent symptoms of hyperviscosity; this has not been necessary for > 18 months since therapy.

Seven patients experienced relief of bone pain within 1 week after starting the drug; it persisted in all responding patients but was transient in the two patients who did not experience either regression or stabilization of disease.

Drug toxicity was minimal, consisting of mild mucositis in 14 patients and a maculopapular rash on exposed areas in three patients. Hematopoietic depression was minimal. Seven patients have died, either from disease progression or, in one patient, from a preleukemic state (median time to death, 5 months). The remaining patients are alive with a median time in the study of 13 months. One patient was lost to followup.

DISCUSSION

In the clinical trial described, PZF produced remarkable tumor regression in only one of 14 patients; however, a second patient has had clear objective and clinically useful response of > 18 months' duration. An additional three patients have had symptomatic relief for > 3 months associated with stabilization of disease lasting up to 11 months. Although the overall PR rate has been low, it is of interest that any therapeutic benefit could be derived in a heavily pretreated group of myeloma patients with an antimetabolite that would be expected to injure only the proliferating cell population.

The principal objective response occurred in one of the three patients in this series who manifested plasmacytomas. Although cell kinetic studies were not done in this patient, other investigators have reported high labeling indices in plasmacytomas in contrast to the low rate of proliferation that generally characterizes myeloma cells in the bone marrow (12).

Although these studies have not demonstrated major therapeutic activity of PZF in heavily pretreated patients with myeloma, the observed effects suggest that PZF merits further trial in this disorder. The chronic nature of myeloma would make it possible to administer PZF for 2–3 months as an initial agent in patients with this disease; nonresponding patients could be transferred promptly to more conventional protocols. It may also be worthwhile to explore the addition of PZF to the treatment programs of patients who have achieved a stable PR but no additional tumor cell kill with the conventional regimens of steroids and alkylating agents.

REFERENCES

1. Sweeney MJ, Davis FA, Gutowski GE, et al. Experimental antitumor activity of pyrazomycin. Cancer Res 33:2619–2623, 1973.
2. Gutowski GE, Sweeney MJ, DeLong DC, et al. Biochemistry and biological effects of the pyrazofurins (pyrazomycins): initial clinical trial. Ann NY Acad Sci 255:544–551, 1975.
3. Ohnuma T, Roboz J, Shapiro ML, et al. Pharmacological and biochemical effects of pyrazofurin in humans. Cancer Res 37:2043–2049, 1977.
4. Cadman EC, Dix DE, and Handschumacher RE. Clinical, biological, and biochemical effects of pyrazofurin. Cancer Res 38:682–688, 1978.
5. Ohnumu T, and Holland JF. Initial clinical study with pyrazofurin. Cancer Treat Rep 61:389–394, 1977.
6. Salem PA, Bodey GP, Burgess MA, et al. A phase I study of pyrazofurin. Cancer 40:2806–2809, 1977.
7. Budman D, Currie V, and Wittes R. Phase II trial of pyrazofurin in malignant melanoma. Cancer Treat Rep 61:1733–1734, 1977.
8. Gralla RJ, Currie VE, Wittes RE, et al. Phase II evaluation of pyrazofurin in patients with carcinoma of the lung. Cancer Treat Rep 62:451–452, 1978.
9. Creagan ET, Rubin J, Moertel CG, et al. Phase II study of pyrazofurin in advanced colorectal carcinoma. Cancer Treat Rep 61:491–493, 1977.
10. Gralla RJ, Sordillo PP, and Magill GB. Phase II evaluation of pyrazofurin in patients with metastatic sarcoma. Cancer Treat Rep 62:1573–1574, 1978.
11. Committee of the Chronic Leukemia-Myeloma Task Force, National Cancer Institute. Proposed guidelines for protocol studies. II. Plasma cell myeloma. Cancer Chemother Rep Part 3, vol 4(1):145–158, 1973.
12. Drewinko B, and Alexanian R. Growth kinetics of plasma cell myeloma. JNCI 58:1247–1253, 1977.
13. Karnofsky D, Abelman W, Craver LF, et al. The use of nitrogen mustards in the palliative treatment of carcinoma. Cancer 1:634–656, 1948.
14. Salmon S. Cell kinetics in multiple myeloma. Arch Intern Med 135:131–138, 1975.

High-Dose Methotrexate in Malignant Melanoma[1]

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SUMMARY

High-dose methotrexate was given to 28 patients with advanced malignant melanoma who had previously failed to respond to two chemotherapy protocols. There were five instances of serious, but not fatal, toxicity. One patient has had an objective response and seven patients have had stabilization of disease with an average duration of 4 months.

[Cancer Treat Rep 63:1405–1407, 1979]

As with other malignancies, clinical research in the chemotherapy for malignant melanoma has followed two directions: (*a*) attempts to develop more efficient combination protocols with agents of established activity and (*b*) trials of relatively new single agents or new schedules of administration of older agents.

DTIC has been one of the most useful single agents in the treatment of malignant melanoma, with a response rate of about 20% and tolerable toxicity in most studies (1–4). The nitrosoureas produce closely similar responses (5–9). Combination protocols have generally not produced higher response rates (10–12).

High-dose methotrexate (HD-MTX) with leucovorin rescue has been found to be effective in malignant melanoma (13). The response rate with monthly HD-MTX is about 20% (14).

The following report describes the experience in 28 patients with disseminated malignant melanoma treated with HD-MTX. All of these patients had failed to respond to at least two previous protocols: one protocol with DTIC alone or DTIC and *Corynebacterium parvum* and one protocol with a nitrosourea (methyl-CCNU plus procarbazine or a combination of BCNU, actinomycin D, and vincristine). Since, in many oncologic centers, DTIC and the nitrosoureas are also used first in the treatment of malignant melanoma, the effect of HD-MTX after failure with these drugs is of particular interest.

MATERIALS AND METHODS

Of the 28 patients treated, there were 18 males and ten females ranging in age from 22 to 72 years (mean, 47.5 years). All of the patients were white. All patients had disseminated tumor. Twenty-two patients had visceral disease while six patients had multiple subcutaneous nodules or recurrent extensive lymph node involvement; of the latter group of six patients, two were given chemotherapy after removal of gross tumor.

Twenty-three patients received 2 g/m^2 of HD-MTX iv, while five patients received 4 g/m^2 of HD-MTX iv repeated every 4 weeks. MTX was given in 1 liter of 5% dextrose/0.33 normal saline with two ampules of sodium bicarbonate ($NaHCO_3$) over 6 hours, followed 2 hours later by 50 mg of leucovorin rescue iv every 6 hours for ten times. Iv hydration was continued for 48 hours at least (3 liters/day) and alkalinization of the urine was sustained (at $\geq$ pH 7.0) with iv and oral $NaHCO_3$. The plasma-MTX level was determined at least 48 hours after infusion.

RESULTS

Two patients exhibited moderate toxic effects and three patients had severe, but not fatal, toxic effects (wbc count $<$ 1000/μl or platelet count $<$ 20,000/μl and/or stomatitis). Two of these patients required no particular therapeutic measures. One of the remaining patients had a low platelet count and gastrointestinal bleeding and required multiple platelet transfusions (treated elsewhere). Another patient experienced early stomatitis while still hospitalized and had high plasma-MTX levels. Leucovorin rescue was maintained in this patient for 10 days. His plasma-MTX level was still 4.3×10^{-7} M 6 days after infusion. His serum creatinine level prior to treatment was 0.7 mg/100 ml, but rose to 4.2 mg/100 ml and declined to 1.7 mg/100 ml prior to discharge, although his BUN remained within normal limits. While his wbc count was 24,200/μl and his platelet count was 257,000/μl before treatment, a nadir of 6600/μl and 86,000/μl, respectively, occurred after treatment. The third patient experiencing severe toxic effects had an MTX-plasma level of 6.9×10^{-6} M at 48 hours and leucovorin rescue was continued for a total of 17 days after MTX infusion. His wbc count

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dropped to 1200/μl and his platelet count to 16,000/μl 11 days after MTX infusion. His serum creatinine rose from a pretreatment value of 0.6 mg/100 ml to 1.5 mg/100 ml after treatment, but his BUN remained within normal limits.

The patients received an average of 2.3 courses. Eleven patients were declared treatment failures after one course due to obvious progression, while eight patients were declared treatment failures after two courses. In one patient, chemotherapy was changed after one episode of toxicity without failure. Seven patients were considered to have stabilization of their disease for > 2 months and they received a minimum of three courses of HD-MTX (range, three to 11 courses; mean, five courses). The mean period of disease stabilization was 4 months. One patient with multiple subcutaneous nodules had $> 50\%$ regression of large nodules and disappearance of smaller nodules. Presently, this response has lasted for 6 months.

Four of the patients are alive. In the group of patients who had disease stabilization and then died the average survival was 7 months from initiation of HD-MTX treatment, while in the patients who failed to respond the average survival was 3 months.

DISCUSSION

HD-MTX with leucovorin rescue has considerable morbidity; five of our 28 patients experienced significant toxicity. Drug-related deaths have been reported (15). However, previous studies have indicated that adequate hydration and alkalinization of the urine reduce this risk. Correlation between plasma-MTX levels and ensuing toxicity has been established (16,17). It has been reported that patients with plasma-MTX levels $> 5 \times 10^{-7}$ M at 48 hours are at risk of developing toxicity (18,19). On the basis of this information, two patients had leucovorin rescue continued for 10 and 15 days, respectively. One patient also received 15 g/day $\times$ 5 days of thymidine as a continuous infusion because of the modulating effect of this drug on MTX toxicity (20–22). These two patients had an elevated serum creatinine after MTX infusion suggesting that the mechanism of toxicity is due, at least in part, to delayed and inefficient excretion because of renal impairment from early MTX precipitation in the renal tubules. However, serum creatinine elevations are not a sensitive index of impending toxicity, particularly elevations of only 25%–50% above baseline (23).

As is well-known, response to different protocols declines in advanced disease. As secondary chemotherapy, the response rate to DTIC or nitrosoureas drops to 0–9% (9). Secondary chemotherapy is associated with a short survival time (mean, 8–14 weeks) (9).

This report of one objective response and seven disease stabilizations in 28 patients with advanced malignant melanoma treated with HD-MTX demonstrates that the response to the latter also drops significantly when it is given after failure with two other chemotherapeutic regimens.

Weekly HD-MTX has been reported to be more effective than monthly administration (14) in malignant melanoma. An improved therapeutic index with HD-MTX has been reported in other tumors (24), although this has been disputed in some reports (25). As has already been advocated (26), a direct comparison between HD-MTX and conventional-dose MTX remains to be made in a randomized trial in malignant melanoma as well as in other sensitive tumors.

REFERENCES

1. Burke PJ, McCarthy WH, and Milton GW. Imidazole carboxamide therapy in advanced malignant melanoma. Cancer 27:744–750, 1971.
2. Luce JK, Thurman WG, Isaacs BL, et al. Clinical trials with the antitumor agent 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (NSC-45388). Cancer Chemother Rep 54:119–124, 1970.
3. Rochlin DB, Wagner DE, Wilson WL, et al. Results of phase I and II studies with imidazole-carboxamide-dimethyltriazeno. (Abstr) *In* Proceedings of the 10th International Cancer Congress, Houston, Tex, 1970. Houston, Tex, Medical Arts Publishing Co, 1970, pp 474–475.
4. Wagner DE, Ramirez G, Weiss AJ, et al. Combination phase I–II study of imidazole carboxamide (NSC-45388). Oncology 26:310–316, 1972.
5. Carter SK, and Newman JW. Nitrosoureas: 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC-409962; BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC-79037; CCNU)—clinical brochure. Cancer Chemother Rep Part 3, vol 1(1):115–151, 1968.
6. DeVita V, and Gold L. Preliminary studies with 1,3 bis(2-chloroethyl)-1-nitrosourea (BCNU). Proc Am Assoc Cancer Res 5:15, 1964.
7. Moon JH. Combination chemotherapy in malignant melanoma. Cancer 26:468–473, 1970.
8. Rall DP, Ben M, and McCarthy DM. BCNU toxicity and initial clinical trial. Proc Am Assoc Cancer Res 4:55, 1963.
9. Ahmann DL. Nitrosoureas in the management of disseminated malignant melanoma. Cancer Treat Rep 60:747–751, 1976.
10. Carter RD, Krementz ET, Hill GJ II, et al. DTIC (NSC-45388) and combination therapy for melanoma. I. Studies with DTIC, BCNU (NSC-409962), CCNU (NSC-79037), vincristine (NSC-67574), and hydroxyurea (NSC-32065). Cancer Treat Rep 60:601–609, 1976.
11. Beretta G, Bonadonna G, Bajetta E, et al. Combination chemotherapy with DTIC (NSC-45388) in advanced malignant melanoma, soft tissue sarcomas, and Hodgkin's disease. Cancer Treat Rep 60:205–211, 1976.
12. Comis RL, and Carter SK. Integration of chemotherapy into combined modality therapy of solid tumors. IV. Malignant melanoma. Cancer Treat Rev 1:285–304, 1974.
13. Eilber FR, and Isacoff W. High dose methotrexate therapy for disseminated malignant melanoma. Proc Am Assoc Cancer Res and ASCO 17:262, 1976.
14. Dufour FR, Eilber FR, and Morton DL. High-dose methotrexate combined with DTIC for metastatic melanoma. Proc Am Assoc Cancer Res and ASCO 19:360, 1978.

15. Von Hoff DD, Penta JS, Helman LJ, et al. Incidence of drug-related deaths secondary to high-dose methotrexate and citrovorum factor administration. Cancer Treat Rep 61:745–748, 1977.
16. Nirenberg A, Mosende C, Mehta BM, et al. High-dose methotrexate with citrovorum factor rescue: predictive value of serum methotrexate concentrations and corrective measures to avert toxicity. Cancer Treat Rep 61:779–783, 1977.
17. Jacobs SA, Dilettuso BA, and Santicky MA. Rescue from high-dose methotrexate (MTX): correlation between 30 hour MTX concentration and dose of leucovorin (LV). Proc Am Assoc Cancer Res and ASCO 18:322, 1977.
18. Isacoff WH, Townsend CM, Jr, Eilber FR, et al. High dose methotrexate therapy of solid tumors: observations relating to clinical toxicity. Med Pediatr Oncol 2:319–325, 1976.
19. Isacoff WH, Eilber F, Tabbarah H, et al. Phase II clinical trial with high-dose methotrexate therapy and citrovorum factor rescue. Cancer Treat Rep 62:1295–1304, 1978.
20. Straw JA, Talbot DC, Tayler GA, et al. Some observations on the reversibility of methotrexate toxicity in normal proliferating tissues. JNCI 58:91–97, 1977.
21. Ensminger WD, and Frei E, III. The prevention of methotrexate toxicity by thymidine infusions in humans. Cancer Res 37:1857–1863, 1977.
22. Ensminger WD, Grindey GB, and Hoglind JA. Antifolate therapy: Experimental approaches using rescue, selective host protection, and drug combinations. Adv Cancer Chemother 1:61–109, 1979.
23. Stoller RG, Drake JC, and Jacobs SA. Monitoring high-dose methotrexate (MTX); value of serum creatinine (Cr) and plasma. Proc Am Assoc Cancer Res and ASCO 17:255, 1976.
24. Levitt M, Mosher MB, DeConti RC, et al. Improved therapeutic index of methotrexate with "leucovorin rescue." Cancer Res 33:1729–1734, 1973.
25. DeConti RC. Phase III comparison of methotrexate with leucovorin vs. methotrexate alone vs. a combination of methotrexate plus leucovorin, cyclophosphamide and cytosine arabinoside in head and neck cancer. Proc Am Assoc Cancer Res and ASCO 17:248, 1976.
26. Catane R, Bono VH, Jr, Louie AC, et al. Letter: High-dose methotrexate, not a conventional treatment. Cancer Treat Rep 62:178–180, 1978.

Host Resistance to Tumor After Cure of Advanced LSA Lymphoma by Treatment With BCNU and Chlorozotocin [1,2]

Brief Reports and Preliminary Communications

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SUMMARY

C57BL mice carrying the syngeneic advanced LSA ascites lymphoma initiated with a 10^6-cell LSA inoculum were treated with four combinations of BCNU and/or chlorozotocin (CLZ) on Days 5 and 6 after tumor inoculation. The percentage of mice surviving ≥ 60 days was > 80% for all four combinations. Surviving mice were then challenged with live LSA cells beginning with 100 cells and increasing the inoculum by factors of 10 every 3 weeks until a 10^7-cell inoculum level was reached. The latter dose was repeated four times. A marked difference in resistance to tumor challenge was observed for the various groups. Surviving tumor-resistant mice at the end of the experiment, classified by original treatment, were as follows: BCNU-BCNU, 86.7%; CLZ-BCNU, 56.3%; BCNU-CLZ, 23.5%; and CLZ-CLZ, 0. Various hypotheses are presented which might explain these differences.

[Cancer Treat Rep 63:1409–1412, 1979]

It has been shown that host resistance to syngeneic tumor challenge is evident using the LSA ascites lymphoma of C57BL mice. Tumor-cured mice or those exposed serially to viable tumor challenges acquire complete resistance to subsequent tumor challenge (1–3). Combinations of BCNU and chlorozotocin (CLZ) can produce a high percentage of cures in C57BL mice with advanced LSA ascites tumors, and appear to confer a resistance to subsequent tumor challenge. Observations on the resistance acquired by drug-cured mice, ie, those surviving > 50 days longer than controls, led us to attempt various treatment schedules in the production of this state of host resistance to tumor challenge. This report describes this effect for the nitrosourea anticancer agents BCNU and CLZ.

MATERIALS AND METHODS

The LSA ascites lymphoma is derived from a spontaneous thymic lymphoma of syngeneic C57BL mice and is causally related to the Rad-LV leukemogenic virus. It is a syngeneic tumor passed at weekly intervals with ascites fluid. No easily detectable immunity is produced by treatment of recipients with killed irradiated tumor cells (2–5), but immunity can be detected by radiation suppression of host resistance (4,6) or by serial sublethal tumor challenge (1).

C57BL mice bred in this laboratory by a strict single-line inbreeding system were used. The mice were 10–13 weeks old at the start of all experiments. They were housed in clean plastic cages and fed Purina chow and water ad libitum. A regular light-dark cycle was used. These mice and the LSA tumor have been carried in this laboratory using strictly inbred animals.

For these experiments, combination chemotherapy trials were set up with large numbers of mice per group, so as to have a sufficient number of survivors which could then be challenged with live LSA cells after they had been "cured" of advanced ascites tumors.

The experimental protocol was as follows: 13-week-old male C57BL mice, bred by strict brother-sister mating in our colony, were injected ip with 10^6 LSA cells. This tumor exhibits a TD50 dose (cell dose necessary to produce tumor takes in 50% of normal recipients) of 1–2 cells (7). Control groups of mice were also set up at the same time; 10^6 tumor cells injected ip caused death in all recipients in about 8.5 days. The rest of the mice were assigned to one of the following treatment schedules (doses of both BCNU and CLZ were 20 mg/kg):

Group 1: BCNU on Days 5 and 6.

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Group 2: CLZ on Day 5 and BCNU on Day 6.
Group 3: BCNU on Day 5 and CLZ on Day 6.
Group 4: CLZ on Days 5 and 6.

After cure of tumor-bearing mice receiving the initial 10^6-cell inoculum, the animals were challenged with ip injected, live LSA cells beginning 2 weeks later. The first inoculum was 100 cells (which is lethal for normal C57BL mice). After 3 weeks the surviving mice were injected with 10^3 live LSA cells, 3 weeks later 10^4 cells were injected, 3 weeks later 10^5 cells were injected, etc, until the 10^7-cell inoculum was reached. The latter cell inoculum was repeated four times at 3-week intervals, and the experiment ended 3 weeks after the last 10^7-cell injection. Mice were observed for tumor development and death. All dead mice were autopsied to confirm the presence of tumor. For every inoculum groups of four to six normal mice were injected with the same number of cells; all of these mice died with ascites tumors at the expected postinoculation time. TD50 assays in normal mice were done once a month during the course of these studies. No changes from the TD50 of 1–2 cells were observed.

RESULTS

The number of cured mice, ie, those surviving 60 days after inoculation with 10^6 cells, for the various treatment groups is shown in table 1. All controls died, with a mean survival time (± SEM) of 8.8 ± 0.3 days. Table 1 shows that BCNU-BCNU treatment had 83.3% survivors, CLZ-BCNU treatment had 88.9% survivors, BCNU-CLZ treatment had 88.5% survivors, and CLZ-CLZ treatment had 83.3% survivors.

On the 63rd day after the initial 10^6-cell inoculum, challenges with ip injected, live LSA cells were initiated as described above. Cells for these challenges came from the regular transplant and they were always harvested and suspended in serum-free Hanks' balanced salt solution. None of the tumor cells used for the subsequent challenges had ever been exposed to BCNU, CLZ, or any other cytotoxic agent.

Figure 1 shows the final results for animals surviving 200 days after the initial posttreatment challenge with viable LSA cells. The highest proportion of mice alive after the various inocula corresponded to the BCNU-BCNU combination, with 86.7% surviving (59.5–98.3).[5] This was followed by the CLZ-BCNU combination, with 56.3% surviving (29.9–80.3)[5]; the BCNU-CLZ combination, with 23.5% surviving (6.81–49.9)[5]; and the CLZ-CLZ combination, with no survivors by the end of the experiment. It was observed (fig 1) that all mice in CLZ-CLZ group were dead by Day 122 after the 100-cell initial challenge.

Skin tests were performed on surviving mice in the BCNU-BCNU group with a cell-derived vaccine prepared from freeze-thawed disrupted LSA cells, or with intradermal injection of 10^4 live LSA cells. Neither of these skin tests led to any reaction at 6, 24, or 72 hours after injection.

TABLE 1.—Survivors after BCNU and/or CLZ therapy on Days 5 and 6 after ip inoculation of 10^6 LSA lymphoma cells

Treatment	No. of mice treated	No. of survivors	% cured
Control	10	0	0
Group 1 (BCNU–BCNU)	18	15	83.3
Group 2 (CLZ–BCNU)	18	16	88.9
Group 3 (BCNU–CLZ)	26	23*	88.5
Group 4 (CLZ–CLZ)	18	15†	83.3

*17 of these mice were used in the present experiment.
†14 of these mice were used in the present experiment.

DISCUSSION

It is clear that mice treated and cured with the combination of BCNU-BCNU were more resistant compared to the CLZ-CLZ group, with the other two groups exhibiting intermediate levels of resistance. The reasons for this are as yet unclear. It is clear, however, that the nitrosoureas were very effective in curing the mice of a large and progressive tumor burden and that the tumor cell-nitrosourea interaction very likely resulted in a damaged cell which retained or amplified its antigenic features. Also, the tumor cell-nitrosourea adduct may have caused delayed death, acting as a potent and prolonged stimulus to the host. It is possible that drug-mediated immunogenic changes, such as those shown by Houchens et al (8) in virus-induced lymphomas, took place immediately after treatment in our experimental system.

Two other possible mechanisms cannot be excluded, although presently they seem less likely: (*a*) the existence of a long-lasting cytotoxic moiety, or (*b*) a mutation mechanism that could trigger and enhance the conventional immune responses of cured mice (9). This may also reflect a change in cell characteristics. One possibility is that a moiety in the nitrosourea molecule (or an intermediate product) interacts with the tumor antigen, probably located in the tumor cell membrane. Since the nitrosoureas are alkylating agents, some membrane reactivity is plausible. This cell adduct somehow unmasked and amplified the tumor antigenicity when the cells were killed by the nitrosoureas. BCNU was found to be much more effective in this regard, and resulted in a greatly enhanced host-resistant state compared to CLZ.

The lower activity of CLZ in enhancing antigenicity may be explained by the glucopyranose ring, which may dominate the antigenic specificity of the whole cell (10–13). Recent work by Killion (13) would seem to support

[5]95% confidence limits.

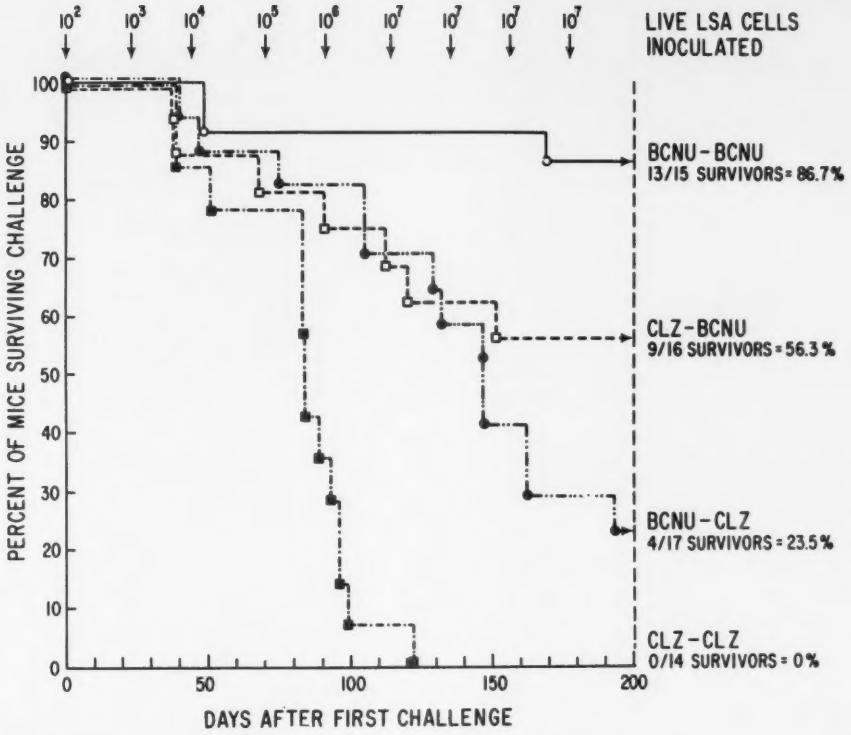

FIGURE 1.—Results in animals surviving 200 days after initial posttreatment challenge with viable LSA cells.

this hypothesis. He has shown that immunotherapy for residual L1210 leukemia results in a higher percent of long-term survivors if it is done with cells either not binding mannosyl-specific columns or eluted from fucose-specific columns compared to cells eluted from galactose-specific columns.

The fact that host resistance to the LSA lymphoma is so difficult to detect has been well-documented (1–6). Nonetheless, this resistance has been reported in other Rad-LV-derived tumors as well as tumor-specific antisera produced against Rad-LV-derived mouse lymphomas (1,3,6,14,15). Killed tumor cells alone are ineffective in producing any host resistance to syngeneic lymphoma (4,16). However, normal host suppression by radiation or steroids (3,17), as well as by serial challenge of mice with small but increasing numbers of viable tumor cells, readily allows this syngeneic tumor resistance to be shown. Kollmorgen et al (18) found that mice cured of L1210 leukemia by BCNU, vibrio cholera neuraminidase, and BCG developed tumor immunity.

The present experiments show that cure of mice bearing advanced tumor using BCNU or CLZ not only can produce a state of complete resistance to further challenges with tumor cells derived from the original challenge tumor, but can also produce markedly different degrees of resistance, presumably depending on the chemical structure of the alkylating agent used.

REFERENCES

1. MARUYAMA Y, and FEOLA JM. Active host resistance for transplant syngeneic C57BL lymphomas. Oncology 28:52–62, 1973.
2. FEOLA J, and MARUYAMA Y. Comparison of chemotherapy, radiation and heat treated cells for host resistance to LSA ascites tumor. *In* Proceedings of the 6th Annual Meeting of the Southeastern Cancer Research Association, Kiawah Island, SC, Nov 15–17, 1978.
3. MARUYAMA Y. Contribution of host resistance to radiosensitivity of an isologous murine lymphoma in vivo. Int J Radiat Biol 12:277–292, 1967.
4. MARUYAMA Y. Recognition of the irradiated isogenic mouse lymphoma cell. Radiology 92:630–632, 1969.
5. MARUYAMA Y, CEMAN C, and MCHUGH RB. Radiation dose-independent inactivation of isologous mouse lymphoma cells by nonspecific resistance. Cancer Res 30:2245–2251, 1970.
6. MARUYAMA Y. Host resistance and radiation response of syngeneic mouse lymphoma cells. JNCI 46:963–971, 1971.
7. FEOLA J. Effects of iodoacetamide and X-rays on a murine ascites tumor. Radiat Res 70:118–124, 1977.

8. Houchens DP, Bonmassar E, Gaston MR, et al. Drug-mediated immunogenic changes of virus-induced leukemia in vivo. Cancer Res 36:1347–1352, 1976.
9. Bonmassar E, Bonmassar A, Vadlamudi S, et al. Immunological alteration of leukemic cells in vivo after treatment with an antitumor drug. Proc Natl Acad Sci USA 66:1089–1095, 1970.
10. Zopf DA, and Ginsburg V. Carbohydrate antigens of cell surfaces. *In* Biology and Chemistry of Eucaryotic Cell Surfaces (Lee EYC, and Smith EE, eds). New York, NY, Academic Press, 1974, pp 259–270.
11. Miller DK, Cooper AM, Brown MC, et al. Reversible loss in suspension culture of a major cell-surface glycoprotein of the TA3-Ha mouse tumor. JNCI 55:1249–1252, 1975.
12. Killion JJ, Wallenbrock MA, Rogers JA, et al. Tumorigenicity and the expression of cell-surface carbohydrates. Nature 261:54–56, 1976.
13. Killion JJ. Immunotherapy with tumor cell subpopulations. I. Active, specific immunotherapy of L1210 leukemia. Cancer Immunol Immunother 4:115–119, 1978.
14. Ferrer JF, and Kaplan HS. Antigenic characteristics of lymphomas induced by radiation leukemia virus (Rad LV) in mice and rats. Cancer Res 28:2522–2528, 1968.
15. Haran-Ghera N. Host-resistance against isotransplantation of lymphomas induced by the radiation leukemia virus. Nature 222:992–993, 1969.
16. Revesz L. Detection of antigeneic differences in isologous host-tumor system by pretreatment with heavily irradiated tumor cells. Cancer Res 20:443–451, 1960.
17. Maruyama Y, and Johnson EA. Quantitative study of isologous tumor cell inactivation and effective cell fraction for the LSA mouse lymphoma. Cancer 23:309–311, 1969.
18. Kollmorgen GM, Sansing WA, Cantrell JL, et al. Treatment of murine lymphocytic leukemia with BCNU, VCN treated cells and BCG. *In* Neoplasm Immunity: Theory and Application (Crispen RG, ed). Chicago, Ill, Institution for Tuberculosis Research, 1975, pp 27–42.

Reinduction Therapy for Adult Acute Leukemia With Adriamycin, Vincristine, and Prednisone: A Southwest Oncology Group Study [1,2,3]

Brief Reports and Preliminary Communications

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SUMMARY

In an attempt to improve remissions and survivals in previously treated patients with adult acute leukemia, we gave Adriamycin, vincristine, and prednisone for induction therapy, followed by 6-mercaptopurine and methotrexate for maintenance therapy to patients attaining complete remission (CR). The study group consisted of 18 patients with acute myeloblastic leukemia (AML), ten with acute lymphoblastic leukemia, and one with acute undifferentiated leukemia. Only one patient had previously received Adriamycin. Overall, there were ten CRs and two partial remissions. The five CRs and one partial remission in patients with AML occurred among those with one prior induction attempt; none of the eight AML patients with more than one prior induction attempt responded. The actuarial median duration of CR was 15 weeks and was similar for AML and acute lymphoblastic leukemia patients. Responders had a longer median survival (30 weeks) than nonresponders (9 weeks). Thus, although a reasonable number of responses in previously treated patients were obtained with this program, improvements in maintenance therapy are clearly needed.

[Cancer Treat Rep 63:1413–1415, 1979]

The anthracycline antibiotics, Adriamycin and daunorubicin, have been recognized for some time as active and useful agents in the management of acute leukemia (1,2). Adriamycin has been particularly favored by the Southwest Oncology Group who have successfully used the drug in combinations for the primary treatment of adult acute leukemia, both acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL) (3). Second remissions in previously treated patients have, however, been reported to be inferior (4,5) and, in an attempt to salvage these patients, a study was designed using a combination of Adriamycin, vincristine, and prednisone for reinduction, and a maintenance regimen of methotrexate and 6-mercaptopurine for patients entering complete remission (CR). Until recently, it has been the practice of the Southwest Oncology Group to manage both AML and ALL patients according to the same chemotherapy protocol.

PATIENTS, MATERIALS, AND METHODS

Twenty-nine patients (13 males and 16 females) with AML (18 patients), ALL (ten patients), and acute undifferentiated leukemia (AUL) (one patient), whose ages ranged from 17 to 71 years, were admitted to the study. All of these patients had received previous chemotherapy in various combinations. All of the ALL-AUL patients and all but three of the AML patients had been previously exposed to vincristine and prednisone. All patients with AML had previously received cytosine arabinoside. Only one patient had previously received Adriamycin. In the induction regimen, Adriamycin (75 mg/m^2 iv) and vincristine (1.4 mg/m^2 iv; maximum dose, 2 mg) were given on Day 1. The same dose of Adriamycin was given on Day 15 and the same dose of vincristine was repeated on Days 8, 15, and 22. Prednisone was administered orally at a dose of 40 mg/m^2 for 28 days and thereafter tapered to 0 over 7 days.

Bone marrow aspiration was performed on Day 28, at which time patients were evaluated for CR and partial remission (PR) by previously described criteria (6). Patients with a PR received further Adriamycin (75 mg/m^2

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iv on Day 29), vincristine (1 mg/m^2 on Days 29 and 36), and prednisone (40 mg/m^2/day orally until Day 42), after which a further bone marrow aspiration was performed.

Patients entering CR received the following maintenance regimen: 6-mercaptopurine (75 mg/m^2/day orally as a single dose) plus methotrexate (15 mg/m^2/week orally as a single dose). Doses were adjusted for hematologic and gastrointestinal toxicity and renal dysfunction. One ALL patient was lost to followup during remission and one ALL patient was placed on a different maintenance regimen.

Statistical methods used included the modified Wilcoxon test for comparison of remission durations and survival (7). All P values cited are two-tailed.

RESULTS

Response Rates

The overall remission rate was 41%, with ten CRs and two PRs. ALL patients responded more frequently (five CRs and one PR, 60%) than those with AML (five CRs and one PR, 33%), but this difference is not significant. Responses in AML patients were confined to those ten patients with one prior induction attempt only. The AML patients entering CR included one with the Ph' abnormality in his marrow but no history of a prior chronic leukemic phase, and one with erythroblastic leukemia.

Remission Duration and Survival

Actuarial median duration of CR was 15 weeks (fig 1) and was almost identical for AML and ALL patients. Actuarial median duration of survival was 13 weeks and was also the same for AML and ALL-AUL patients. Responders survived longer than nonresponders ($P < 0.02$, fig 2).

Toxicity

All patients had hematologic toxicity, seven had neuropathy (mild, one patient; moderate, four patients; and severe, two patients), and six had gastrointestinal toxicity, including stomatitis (mild, two patients; moderate, two patients; and severe, two patients). There was no cardiac toxicity, although one patient noted mild chest pain.

DISCUSSION

Despite the obvious importance of treatment following relapse in determining survival of adult acute leukemia patients, little data have been previously presented, except for results of studies with new agents. This study indicates that second remissions can be obtained for an appreciable percentage of patients. Among patients with ALL, the CR rate of 50% noted here was not surprisingly high considering the known single-agent response rates with the agents used. The CR rate was slightly, but not significantly, lower than that noted when the identical regimen (8) was given to previously untreated adult patients (72%). This was presumably due, in part, to prior exposure to regimens including vincristine and prednisone and consequent drug resistance. The response rate in AML patients, particularly the group with one prior

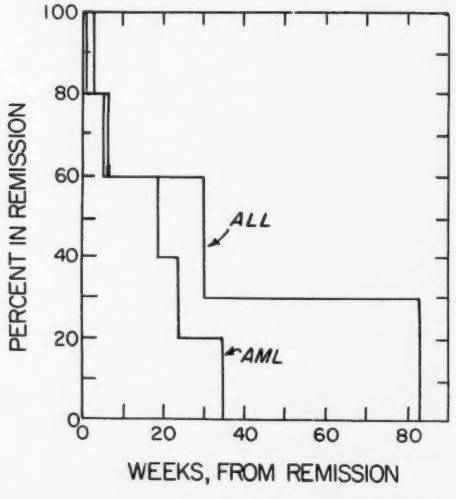

FIGURE 1.—Actuarial duration of CR in AML and ALL patients. A single patient, who was lost to followup during remission, is indicated as a censored point at 6 wks.

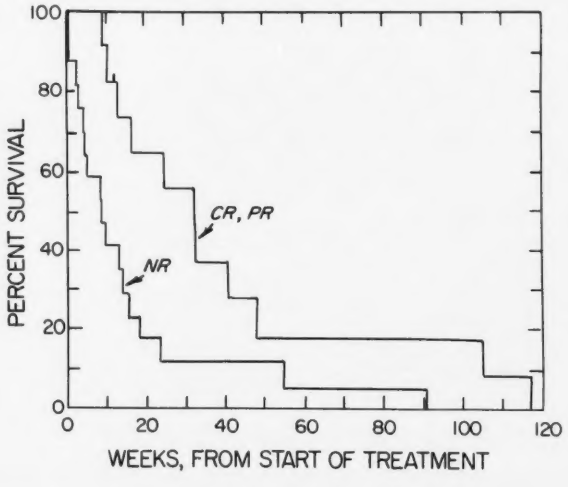

FIGURE 2.—Actuarial duration of survival of responders (CR and PR) compared to nonresponders ($P < 0.02$).

induction attempt, was perhaps slightly higher than might have been expected from the single-agent response to anthracyclines (1,2,9,10). Although vincristine and prednisone have little single-agent activity in AML (10), it is possible that they add some benefit. Other combinations designed specifically for AML may have greater effectiveness, even in previously treated patients. We therefore do not recommend that adult AML and ALL should, ordinarily, be treated identically.

The durations of response and survival were disappointingly short in this series (figs 1 and 2) and were identical for AML and ALL patients. Survival was slightly superior for responders compared to nonresponders (fig 2).

Although the results of this study are encouraging, insofar as they reveal the activity of Adriamycin, vincristine, and prednisone in successful reinduction of adult acute leukemic patients, optimism is curbed by the short duration of remission. The latter emphasizes the need to develop regimens which will prolong first remission duration. There are already indications that the addition of Adriamycin to cytosine arabinoside, vincristine, and prednisone may, to a large extent, achieve this aim (3). Clearly, there is a need also for the development of better reinduction and maintenance therapy for these patients when they relapse.

REFERENCES

1. Dreyfus B, Sultan C, Boiron M, et al. Sur le traitement d'attaque par la rubidomycine de 19 cas de leucémia aigue myéloblastique. Nouv Presse Med 76:55–57, 1968.
2. Mathé G, Amiel JL, Hayat JM, et al. Essai de l'adriamycine dans le traitement des leucémies aigues. Nouv Presse Med 78:1997–1999, 1970.
3. Freireich EJ, Keating MJ, Gehan EA, et al. Therapy of acute myelogenous leukemia. Cancer 42:874–882, 1978.
4. Rodriguez V, Bodey GP, McCredie KB, et al. Combination 6-mercaptopurine-Adriamycin in refractory adult acute leukemia. Clin Pharmacol Ther 18:462–466, 1975.
5. Embury SH, Elias L, Heller PA, et al. Remission maintenance therapy in acute myelogenous leukemia. West J Med 126:267–272, 1977.
6. Hewlett JS, Battle JD, Jr, Bishop RC, et al. Phase II study of A-8103 (NSC-25154) in acute leukemia in adults. Cancer Chemother Rep 42:25–28, 1964.
7. Gehan EA. A generalized Wilcoxon test for comparing arbitrarily singly-censored samples. Biometrika 52:203–223, 1965.
8. Shaw MT, and Raab SO. Adriamycin in combination chemotherapy of adult acute lymphoblastic leukemia: a Southwest Oncology Group study. Med Pediatr Oncol 3:261–266, 1977.
9. Von Hoff DD, Rozencweig M, and Slavik M. Daunomycin: an anthracycline antibiotic effective in acute leukemia. Adv Pharm Chem 15:1–50, 1978.
10. Henderson ED. Acute myelogenous leukemia. *In* Hematology, 2nd ed (Williams WJ, Beutler E, Erslev AJ, et al, eds). New York, NY, McGraw-Hill, 1977, chap 92, pp 830–840.

Combination Chemotherapy for Extracranial Metastases of a Primary Malignant Cerebral Neoplasm [1,2]

Brief Reports and Preliminary Communications

Martin W. Oster [3,*]

SUMMARY

A patient with a mixed malignant glioma metastatic to the bones and lungs failed to respond to two different broad-spectrum combination chemotherapy regimens which included Adriamycin, methotrexate, cyclophosphamide, CCNU, and *cis*-dichlorodiammineplatinum(II).

[Cancer Treat Rep 63:1417–1418, 1979]

Extracranial metastases of primary malignant tumors of the brain are rare (1). Most reported cases have suggested an association between prior craniotomy and subsequent extracranial dissemination of the cerebral tumor, although several case reports of extracranial metastases in the absence of previous craniotomy have appeared (2).

There appears to be virtually no information available in the literature on the sensitivity of extracranial metastases of cerebral malignancies to systemic chemotherapy. This is particularly unfortunate since information regarding chemotherapy for primary cerebral neoplasms may be only partly relevant for their extracranial metastases. This is so because the inability of systemically administered chemotherapeutic agents to reach primary brain tumors is totally irrelevant in the chemotherapy for extracranial metastases of these same malignancies. Agents ineffective for intracranial neoplasms may be quite useful for their extracranial metastases.

This report presents a case of extracranial metastases of a primary malignant cerebral tumor which over several years demonstrated increasingly malignant histology. Several chemotherapeutic agents were used without success in controlling the growth of the metastatic lesions. Although the treatment failed, it is hoped that this report will prompt others to report their experiences with chemotherapy for such extracranial metastases so that a data base can be created in the literature for dealing with this unusual situation.

CASE REPORT

In May 1973, a 27-year-old man presented with severe right-sided headaches, diplopia, bilateral papilledema, and left homonomous hemianopia. A right occipital-parietal brain tumor was diagnosed and resected, with pathology showing a low-grade neoplasm suggestive of an ependymoma. The patient received postoperative whole-brain radiotherapy and did well until October 1977 when he developed headaches, lethargy, left homonomous hemianopia, and left-sided weakness. A large recurrent tumor was documented and removed surgically, with pathology showing features of an oligodendroglioma. Further recurrent tumor was removed in May 1978, with pathology showing a mixed malignant glioma with ependymal, oligodendroglial, and astrocytic features. In August 1978 he developed a productive cough without hemoptysis, dyspnea on exertion, left pleuritic chest pain, and lethargy without neurologic abnormalities. A chest roentgenogram revealed multiple bilateral metastatic nodules. Although bone roentgenograms were normal, bone scan revealed multiple areas of increased uptake in many ribs, the right clavicle, the right humeral head, the left femoral neck, and several thoracic and lumbar vertebral bodies. Liver function tests showed markedly elevated levels and liver scan revealed diffuse hepatomegaly without any discrete filling defects. Cbc, serum calcium, and intravenous pyelogram were normal. Biopsy of the right clavicle revealed bone and bone marrow invaded by a malignant glioma that appeared similar to but more malignant than the three prior brain tumor specimens of 1973, 1977, and 1978. The patient was treated with a combination chemotherapy regimen consisting of methotrexate (30 mg/m^2), Adriamycin (35 mg/

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m^2), cyclophosphamide (400 mg/m^2 iv), and CCNU (30 mg/m^2 orally) given monthly for two cycles. Before a third cycle was administered in November 1978, the patient was re-evaluated for response. His clinical status was worse, with persistent cough and dyspnea, weakness, anorexia, and cachexia. A chest roentgenogram showed an increase in the size and number of metastatic nodules in both lungs compared to his previous roentgenograms. Liver function tests and liver scan were unchanged from his prior studies. Skeletal roentgenograms, previously normal in August 1978, now demonstrated multiple areas of osteoblastic, metastatic disease in the spine, ribs, pelvis, and elsewhere. His chemotherapy was changed to Adriamycin (60 mg/m^2 iv) and *cis*-dichlorodiammineplatinum(II) (60 mg/m^2 iv) which he received on November 17, 1978, and December 15, 1978. However, his condition continued to deteriorate clinically, with progressive lung metastases demonstrated on chest roentgenogram. He died on December 23, 1978. Permission for an autopsy was denied.

DISCUSSION

This case is another example of the rare phenomenon of extracranial metastases of a malignant primary brain tumor. As is usually the case, the metastases occurred in a patient with a history of previous craniotomy. Metastases were quite extensive in this case, involving many bones, the lungs, and probably the liver. Of interest in this case was the progressive increase from 1973 to 1978 in malignant histologic characteristics of the tumor, a phenomenon known to occur in cerebral tumors. Because of the lack of information in the literature concerning chemotherapy for extracranial metastases of cerebral tumors, the patient was initially treated with a regimen which was felt to have a wide spectrum of antitumor activity. This regimen was unfortunately ineffective, as was a second regimen containing *cis*-dichlorodiammineplatinum(II).

Experience with chemotherapy for malignant gliomas has not been overly successful (3). Only a few chemotherapeutic agents have demonstrated much activity. Results of combinations of these agents have likewise not been particularly impressive. Although the blood-brain barrier has been cited as the reason why many systemically administered chemotherapeutic agents are ineffective for cerebral neoplasms, it has recently been proposed that this is not so. Since brain tumors do not possess a tight capillary structure, Levin has postulated that the blood-brain barrier is not a major obstacle in brain tumor chemotherapy (4). Rather, he has suggested that the problem is one of drug delivery and attainment of therapeutic drug levels in the brain tumor. Intrinsic tumor insensitivity to drug therapy has also been suggested. There is no doubt that multiple factors are involved. It is clear, however, that in both experimental tumor models and in patients chemotherapeutic agents active against extracranial malignancies are inactive against the same neoplasms when they metastasize to the brain. This disparity in the response of extracerebral and intracerebral tumors suggests that decisions regarding treatment of extracranial metastases of cerebral neoplasms cannot be based simply on knowledge gained from treatment of intracranial cerebral tumors.

Other than passing reference to a response of unbiopsied subcutaneous nodules to vincristine (5), I have not found any reported experiences of chemotherapy for extracranial metastases of cerebral tumors in the medical literature. For that reason, this report, although negative, is presented. Only when other such experiences, hopefully more effective, are recorded will a data base be available for therapeutic decisions in such unusual situations. The extracranial location of these metastases might also shed further light on the intrinsic sensitivity of brain tumors to chemotherapeutic agents. This, coupled with better systems of delivery of these therapeutic agents to intracerebral tumors, might help in the management of cerebral tumors that have not metastasized as well as those that have metastasized outside of the central nervous system.

REFERENCES

1. Ellsworth A. Why do gliomas not metastasize? Arch Neurol 33:73–75, 1976.
2. Terheggen HG, and Müller W. Extracerebrospinal metastases in glioblastoma. Case report and review of the literature. Eur J Pediatr 124:155–164, 1977.
3. Levin VA, and Wilson CB. Brain tumors: chemotherapy—the agents in current use. Semin Oncol 2:63–67, 1975.
4. Levin VA. A pharmacologic basis for brain tumor chemotherapy. Semin Oncol 2:57–62, 1975.
5. El-Gindi S, Salama M, El-Henawy M, et al. Metastases of glioblastoma multiforme to cervical lymph nodes. J Neurosurg 38:631–638, 1973.

Phase II Evaluation of ThioTEPA for Treatment of Central Nervous System Tumors [1,2]

Brief Reports and Preliminary Communications

Michael S. Edwards,* Victor A. Levin, Margaret L. Seager, Tana L. Pischer, and Charles B. Wilson [3,4]

SUMMARY

Nineteen patients with primary malignant gliomas were treated with thioTEPA. It was found to be ineffective in 13 evaluable patients.

[Cancer Treat Rep 63:1419–1421, 1979]

ThioTEPA was evaluated in a phase I–II trial for recurrent primary malignant gliomas. Although only anecdotal information exists to document its effectiveness in treating primary intracranial neoplasms (1–5), activity has been noted against central nervous system metastases from breast carcinoma (6–8) and against meningeal leukemia and carcinomatosis (9,10). Because thioTEPA is highly lipid-soluble, which should allow passage across the blood-brain barrier, and is an alkylating agent, we designed the clinical trial reported here.

MATERIALS AND METHODS

Patient selection.—Nineteen patients were entered into this study, 18 of whom had histologically confirmed primary malignant brain tumors. One patient had a presumed malignant pineal region glioma (table 1). Patients ranged from 18 to 64 years of age (median, 41 years). All had received radiation therapy and 13 of 19 had received chemotherapy. At the time thioTEPA therapy was started, all patients had evidence of tumor recurrence following radiation therapy and/or chemotherapy. Recurrence was documented by radionuclide (RN) brain scan, computerized tomographic (CT) brain scan, and clinical neurologic examination. Eligibility requirements included no surgery for at least 2 months, no radiation therapy for at least 3 months, no chemotherapy for at least 6 weeks, and normal results of a cbc, platelet count, SMA-12 panel, urinalysis, and prothrombin time. Informed consent was obtained from each patient (and/or responsible next-of-kin) before therapy was begun.

Drug administration.—ThioTEPA was given in doses ranging from 15 to 60 mg/m^2 iv once every 3 weeks. If a patient did not demonstrate marrow toxicity, the thioTEPA dose was increased by 5–10 mg/m^2/dose.

Patient evaluation.—After two courses of thioTEPA therapy, patients were evaluated at 6-week intervals with a neurologic examination, RN brain scan, and CT brain scan. The results of these tests were compared with results from the most recent preceding tests (11). *Response* was defined as definite improvement in at least two of the three parameters in the same or consecutive evaluations, provided that the patient was receiving a stable or decreasing dose of steroids. *Progression* was defined as definite deterioration in at least two of the three parameters, in the same or consecutive evaluations, provided that the steroid dose had not been decreased. *Stable disease* was defined as no change in the three parameters or a definite change in only one of the three.

RESULTS

Twelve of the 19 patients in this study had nonglioblastoma malignant gliomas, six patients had glioblastoma multiforme, and one patient had an oligodendroglioma. Six of the 19 patients were unevaluable for response for the following reasons: the oligodendroglioma patient because of a lack of documented recurrence; one patient because of concomitant radiation therapy; and four patients because of death before receiving two doses of thioTEPA. Sixteen patients were considered evaluable for drug toxicity.

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[4]We are deeply indebted to neurosurgeons in private practice and at the Kaiser-Permanente Medical Centers of the Greater Bay Area for referring patients to us. We thank Neil Buckley for editorial assistance.

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TABLE 1.—Response of patients to thioTEPA chemotherapy

Tumor type	Patient No.	Previous chemotherapy	ThioTEPA					
			No. of courses	Dose (mg/m^2)	Toxicity levels*		Response to therapy	Wks on study
					Wbcs	Platelets		
Glioblastoma multiforme	1	1 BCNU × 1	1	45 × 1	0		Not evaluable	5 (died)
	2	2 BCNU × 5, 1 VM-26 × 6	2	25 × 1, 30 × 1	2	2	Progression	8
	3	1 BCNU × 5	2	30 × 2	2	4	Progression	6 (died)
	4	1 BCNU × 4	2	25 × 2	4	1	Progression	10
	5	1 BCNU × 7	3	15 × 1, 35 × 2	3	3	Progression	—
	6	1 BCNU × 7	4	25 × 2, 35 × 1, 45 × 1	2	3	Progression	15 (died)
Anaplastic astrocytoma	7	PCV§ × 2	1	40 × 1	0		Not evaluable	Died after 1 wk
	8	—	4	40 × 4	0		Stable	12
Malignant glioma	9	—	1	40 × 1	2		Not evaluable	Died after 2 wks
	10	4 hydroxyurea, 1 VM-26, 1 methyl-CCNU × 4, 1 procarbazine × 1	1	25 × 1	0		Not evaluable	3
	11	1 methyl-prednisolone × 7	1	45 × 1	0	1	Not evaluable	5 (died)
	12	—	2	45 × 1, 35 × 1	0	1	Progression	6
	13	1 BCNU × 5	2	30 × 2	2	1	Progression	6
	14†	2 PCV§ × 6, 2 BCNU/5-FU × 4	2	14.7 × 2	3		Progression	4
	15†	2 BCNU/5-FU × 8, 1 hydroxyurea × 1	2	35 × 2		4	Progression	6
	16†	—	2	45 × 2	3		Stable	10
Gemistocytic astrocytoma	17	—	4	60 × 2, 45 × 2	0		Not evaluable, concomitant radiotherapy	13
Mixed malignant glioma	18	1 hydroxyurea × 1	4	30 × 2, 50 × 2	0		Stable	14
Oligodendroglioma	19	—	3	45 × 3	3	3	Stable‡	12

*Toxicity levels:

Level	*Wbcs/mm³*	*Platelets/mm³*
0	≥4000	≥125,000
1	3000–3999	100,000–124,999
2	2000–2999	75,000–99,999
3	1500–1999	50,000–74,999
4	<1500	<50,000

†Originally diagnosed as low-grade gliomas; malignancy evident at time of treatment with thioTEPA on basis of neuroradiologic studies and clinical deterioration.

‡Positive recurrence not documented; evaluability of this case is questionable.

§PCV = procarbazine, CCNU, and vincristine.

Response to therapy.—Two or more doses of thioTEPA were administered to 12 patients evaluable for response, none of whom responded to therapy. Four patients showed stable disease for 10, 12, 12, and 14 weeks. However, two patients had received a significant escalation of their corticosteroid dose during that period and none showed improvement in either the RN or CT brain scans.

Toxicity.—The major toxicity exhibited was hematologic. An occasional patient experienced nausea and vomiting that lasted < 2–4 hours. Table 1 defines the hematologic toxicity level produced during therapy. The toxicity exhibited for patients receiving thioTEPA as their first form of chemotherapy was level 1 or less in 78% of the patients. However, during the third and fourth courses, toxicity to level 3 was seen in 50% of the patients. As expected, patients receiving thioTEPA as a second, third, or fourth mode of chemotherapy exhibited more severe myelosuppression and were more likely to experience prolonged suppression of both wbcs and platelets. Because of tumor progression, none of the patients received more than four courses.

DISCUSSION

When used in a phase I–II setting for recurrent cerebral neoplasms, no responses were seen in 13 evaluable patients receiving thioTEPA as their first or subsequent form of chemotherapy. We do not feel that a response might be seen at higher drug doses because our patients exhibited significant and often prolonged myelosuppression of both wbc and platelet elements at the doses administered. These data are insufficient to make any statement regarding cross-resistance to prior nitrosourea therapy. This study documents the ineffectiveness of thioTEPA for patients with recurrent primary malignant central nervous system neoplasms.

REFERENCES

1. Mark VA, Kjellburg RN, Ojemann RG, et al. Treatment of malignant brain tumors with alkylating agents. Neurology 10:772–776, 1960.
2. Aronson HA, Flanigan S, and Mark JBD. Chemotherapy of malignant brain tumors using regional perfusion. Ann Surg 157:394–399, 1963.
3. Broder LE, and Rall DP. Chemotherapy of brain tumors. Prog Exp Tumor Res 17:373–399, 1972.
4. Deen MR, Newton KA, and Swann GF. Percutaneous intra-arterial chemotherapy in the treatment of intracranial neoplasms. A review of 36 cases. Br J Radiol 40:828–833, 1967.
5. Bateman JC. Chemotherapy of solid tumors with triethylene thiophosphoramide. N Engl J Med 252:879–887, 1955.
6. Davis PL, and Shumway MH. Thio-tepa in the treatment of metastatic cerebral malignancy. JAMA 175:714–715, 1961.
7. Greenspan EM. Clinical Cancer Chemotherapy. New York, NY, Raven Press, 1975.
8. Greenspan EM, Feebin M, Usnick G, et al. Response of advanced breast carcinoma to the combination of the antimetabolite methotrexate and the alkylating agent thio-tepa. Mt Sinai J Med 30:246–267, 1963.
9. Gutin PH, Weiss HD, Wiernik PH, et al. Intrathecal *N,N′,N″*-triethylenethiophosphoramide [thio-TEPA (NSC 6396)] in the treatment of malignant meningeal disease: phase I–II study. Cancer 38:1471–1475, 1976.
10. Gutin PH, Levi JA, Wiernik PH, et al. Treatment of malignant meningeal disease with intrathecal thioTEPA: a phase II study. Cancer Treat Rep 61:885–887, 1977.
11. Levin VA, Crafts DC, Norman DM, et al. Criteria for evaluating patients undergoing chemotherapy for malignant brain tumors. J Neurosurg 47:329–335, 1977.

Phase II Trial of Pyrazofurin, Alone and in Combination With Trifluorothymidine, in Non-Hodgkin's Lymphoma [1,2,3]

Brief Reports and Preliminary Communications

Raymond P. Warrell, Jr, Violante Currie, Sanford Kempin, and Charles Young [4,*]

SUMMARY

A phase II trial of pyrazofurin, alone and in combination with trifluorothymidine, was carried out in patients with advanced non-Hodgkin's lymphoma. None of the 19 patients evaluable for response had complete or partial remissions but 14 had minor regressions. Toxicity, consisting primarily of myelosuppression and stomatitis, was of moderate severity. These studies suggest that pyrazofurin, alone or in combination with trifluorothymidine, is of limited utility in advanced non-Hodgkin's lymphoma.

[Cancer Treat Rep 63:1423–1425, 1979]

Pyrazofurin (PZF) is a new C-ribonucleoside antibiotic which has demonstrated antiviral and antitumor activity in preclinical studies (1). Several studies in man have reported only minor activity in acute myelocytic leukemia (2), sarcoma (3), and carcinomas of the lung (4) and colon (5). Acceptable hematologic toxicity has been produced at weekly doses of 5–7 mg/kg (200–300 mg/m^2) (3,4).

PZF blocks the de novo pathway of pyrimidine synthesis through inhibition of orotidylate decarboxylase; as a consequence of this block, the intracellular uptake of exogenous pyrimidines via a "salvage" pathway is increased (6). This suggests that a synergistic antitumor effect might be achieved by the combined use of PZF with pyrimidine analogs; several studies have recently demonstrated this synergy in vitro using a variety of cell lines (7–9). Therefore, a phase II trial in non-Hodgkin's lymphoma using PZF alone was instituted, followed by a study using this drug in combination with the pyrimidine analog, trifluorothymidine (F_3TdR), in an effort to enhance its activity; the latter agent was chosen because of its lack of mucosal toxicity and studies suggesting that it has some activity in hematologic neoplasms (10).

MATERIALS AND METHODS

Twenty-one patients with advanced non-Hodgkin's lymphoma and objectively measurable disease (all heavily pretreated and refractory to conventional combination chemotherapy) were entered in this study between January 1976 and July 1978. Eleven patients received PZF alone: three with diffuse histiocytic lymphoma (DHL), four with diffuse poorly differentiated lymphocytic lymphoma (DPDL), and four with nodular poorly differentiated lymphocytic lymphoma (NPDL); all are evaluable for response. Six of these patients were subsequently advanced into the combined trial after completion of the single-agent study. A total of 16 patients received PZF combined with F_3TdR; 14 are evaluable for response: eight with DHL, three with DPDL, one with diffuse well-differentiated lymphocytic lymphoma (DWDL), and two with mycosis fungoides. Two patients receiving the combination are not considered evaluable for response: one died of progressive disease prior to completion of the first course of F_3TdR and the other developed stomatitis by the second day and withdrew from the study. This last patient had been heavily pretreated with PZF as a single agent, but he is included in the toxicity analysis of both regimens.

PZF was administered by rapid iv infusion; when given as a single agent, the initial dose was 5 mg/kg weekly, with escalation by 1 mg/kg/week until hematologic toxicity was reached. An adequate trial for PZF alone consisted of a minimum of two injections which produced hematologic toxicity; the mean number of injections per

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[3]Pyrazofurin was supplied by Eli Lilly and Co, Indianapolis, Ind. Trifluorothymidine was supplied by the Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

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patient was 5.3. For patients receiving the combination, PZF was administered without escalation at 5 mg/kg on Day 1 and F_3TdR was administered at 5 mg/kg/day on Days 1–5; the therapy was repeated every 3 weeks. An adequate trial for the combination consisted of at least one full course which achieved hematologic toxicity. In both regimens, the doses of PZF and F_3TdR were each attenuated by 1 mg/kg for a wbc count of 2000–3500/mm^3 or a platelet count of 150,000–200,000/mm^3 and by 2 mg/kg for a wbc count of 2000–2999/mm^3 or a platelet count of 100,000–149,999/mm^3. Both drugs were stopped for a minimum of 1 week for blood cell counts below these levels or if the patient was unable to eat because of stomatitis.

Objective responses were defined as follows: *partial remission (PR)*—a ≥ 50% decrease in the sum of the products of the largest perpendicular diameters of all measurable disease without the appearance of new disease for > 4 weeks; and *minor regression*—an objective decrease (> 25%) in the sum of the products of the largest perpendicular tumor diameters but less than that required for a PR.

TABLE 1.—Major toxic effects of PZF-F_3 TdR

Toxic effect	No. of patients	
	PZF	PZF-F_3TdR
Wbc count (cells/mm^3)		
3000–3500	1	3
2000–2999	2	1
<2000	1	6
Platelet count (cells/mm^3)		
100,000–149,000	4	2
50,000–99,999	5	4
<50,000	2	4
Hemoglobin decrease (g/dl)		
0–1	3	4
1.1–3.0	2	4
>3.0	6	3
Stomatitis		
Mild	3	2
Moderate	1	6
Total No. of patients evaluable for each trial	11	15

RESULTS

There were no PRs in the 19 evaluable patients receiving either PZF alone or PZF in combination with F_3TdR. Minor regressions were seen in five of the 11 patients treated with PZF as a single agent (two with DHL and three with DPDL); nine of the 14 evaluable patients treated with the combination of PZF-F_3TdR showed minor regression (four with DHL, two with DPDL, one with DWDL, and two with mycosis fungoides). Of the six patients pretreated with PZF alone and showing a minor regression (two patients) or stable disease for 2 months (four patients), four demonstrated an additional minor regression when F_3TdR was added in combination.

There was moderate toxicity associated with this treatment, with one probable drug-related death from sepsis after the second course of PZF-F_3TdR during a period of neutropenia (total wbc count = 1700/mm^3). The major toxic effects (table 1) were stomatitis, leukopenia, thrombocytopenia, and anemia ("moderate" stomatitis = patient unable to eat, drug withheld for 1 week). Minor toxic effects from PZF included nausea and vomiting (six patients) and skin rash (two patients); the only additional toxic effects seen with the combined use of PZF-F_3TdR were fever and chills following injections of F_3TdR (two patients).

DISCUSSION

Although PZF showed only minimal activity as a single agent, given the drug's in vitro capability of increasing pyrimidine uptake and recent studies demonstrating synergistic effects when combined with pyrimidine analogs, it was hoped that the PZF-F_3TdR regimen might produce an enhanced antitumor effect by exploiting this biochemical phenomenon. However, while the activity of PZF may have been marginally improved, all responses seen in both of these studies were truly of a minor nature. It remains to be seen whether the in vitro promise of such drug combinations can be translated into improved clinical results, but we conclude that PZF alone, or in combination with F_3TdR, is of limited therapeutic utility in advanced non-Hodgkin's lymphoma.

REFERENCES

1. SWEENEY MJ, DAVIS FA, GUTOWSKI GE, ET AL. Experimental antitumor activity of pyrazomycin. Cancer Res 33:2619–2633, 1973.
2. VOGLER WR, and TRULOCK PD (Writing Committee for the Southeastern Cancer Study Group). Phase I study of pyrazofurin in refractory acute myelogenous leukemia. Cancer Treat Rep 62:1569–1571, 1978.
3. GRALLA RJ, SORDILLO PP, and MAGILL GB. Phase II evaluation of pyrazofurin in patients with metastatic sarcoma. Cancer Treat Rep 62:1573–1574, 1978.
4. GRALLA RJ, CURRIE VE, WITTES RE, ET AL. Phase II evaluation of pyrazofurin in patients with carcinoma of the lung. Cancer Treat Rep 62:451–452, 1978.
5. CREAGAN ET, RUBIN J, MOERTEL CG, ET AL. Phase II study of pyrazofurin in advanced colorectal carcinoma. Cancer Treat Rep 61:491–493, 1977.
6. PLAGEMANN PGW, and BEHRENS M. Inhibition of de novo pyrimidine nucleotide and DNA synthesis and growth of

Novikoff rat hepatoma cells and other cell lines by pyrazofurin (NSC 143095). Cancer Res 36:3809–3812, 1976.
7. Cadman EC, Dix DE, and Handschumacher RE. Clinical, biological, and biochemical effects of pyrazofurin. Cancer Res 38:682–688, 1978.
8. Plagemann PGW, Behrens M, and Abraham D. Metabolism and cytotoxicity of 5-azacytidine in cultured Novikoff rat hepatoma and P388 mouse leukemia cells and their enhancement by preincubation with pyrazofurin. Cancer Res 38:2458–2466, 1978.
9. Cadman E, Eiferman F, Heimer R, et al. Pyrazofurin enhancement of 5-azacytidine antitumor activity in L5178Y and human leukemia cells. Cancer Res 38:4610–4617, 1978.
10. Helson L, Yagoda A, McCarthy M, et al. Clinical trials with trifluoromethyl 2′ deoxyuridine (F_3TdR). Proc Am Assoc Cancer Res 11:35, 1970.

LETTERS

New Regimen of Combination Chemotherapy (CHVV) in the Treatment of Advanced Bladder Cancer: A Pilot Study

We have employed the combination chemotherapy program of CHVV (cyclophosphamide, hydroxyurea, vinblastine, and vincristine) in a phase II study of many types of advanced-stage solid tumors (1). We report here results of CHVV treatment in a group of 19 patients with advanced bladder cancer. The rationale for this polychemotherapy program includes the use of a cycle-nonspecific alkylating drug (cyclophosphamide) followed by an S-phase-specific drug (hydroxyurea) and then by two mitotic spindle-inhibiting drugs (vinblastine and vincristine) with different major toxic effects. Cyclophosphamide (2), hydroxyurea (2), and vincristine (3) have been demonstrated to have single-agent activity in advanced bladder cancer.

The treatment was as follows: cyclophosphamide, 3 mg/kg iv twice daily on Days 1 and 2; hydroxyurea, 50 mg/kg orally daily on Days 3–6, Days 7 and 8, and then rest; vinblastine, 0.1 mg/kg iv on Day 9; and vincristine, 0.01 mg/kg iv on Day 9. This 9-day course of therapy causes a nadir of leukopenia on the 13th day. Myelosuppression may persist to the 21st day. Therefore, CHVV was administered in monthly courses until progression of disease was demonstrated.

The median age of the patients was 62 years (range, 36–81). In all patients the disease was progressing but the life expectancy was > 2 months. Mean performance status (according to Karnofsky) was 60% (range, 40%–80%). None of the patients had undergone any oncologic treatment for at least 1 month. Their bone marrow, hepatic, and renal functions were normal.

At initiation of cytotoxic treatment three of the 19 patients had only inoperable primary tumors ($T_4N_2M_0$), six had only local recurrences, and ten had disseminated metastases (lung, one patient; local recurrence plus lymph nodes, two; local recurrence plus skin and bone, two; lymph node, one; skin, one; lung plus bone, one; and bone only, two). Sixteen patients had undergone partial or radical surgery, five had received radiotherapy, three had received chemotherapy, and two had never been treated. Patients were evaluated for response after the first two courses of treatment; during the second treatment course vinblastine and vincristine were withheld in seven of the 19 patients due to the onset of leukopenia on the 8th day.

Objective evaluation was impossible in one of the 19 patients (cystoscopic investigation after the treatment was not possible). Objective criteria of response were as follows: partial remission (> 50% decrease in measurable lesions for at least 1 month); no change (no appreciable change or < 50% decrease in measurable lesions); and progression (increase of measurable lesions or appearance of new lesions).

Subjective remission was considered the disappearance of or significant decrease of the following symptoms: anorexia, pain, dysuria, walking impairment, and genitourinary bleeding.

Before and after each cycle of treatment patients were evaluated by clinical examination (bimanual evaluation for primary tumors and local recurrences), roentgenographic and cystoscopic evaluation as indicated, and hematologic tests.

TABLE 1.—Clinical toxicity

Toxic effect	No. of patients with toxic effect (%): total patients = 19	
	1st course	2nd course
Anemia	1 (5)	1 (5)
Leukopenia	18 (95)	18 (95)
Thrombocytopenia	1 (5)	1 (5)
Nausea	3 (16)	4 (21)
Vomiting	4 (21)	5 (26)
Diarrhea	1 (5)	0
Stomatitis	1 (5)	1 (5)
Asthenia	1 (5)	1 (5)
Anorexia	1 (5)	0

TABLE 2.—Leukopenia

Wbc count (cells/mm³)	No. of patients with leukopenia (%): total patients = 18	
	1st course	2nd course
4000–3000	2 (11)	4 (22)
3000–1500	13 (72)	9 (50)
< 1500	3 (17)	5 (28)
Median wbc nadir (cells/mm³)	2000 (range, 600–3600)	2100 (range, 600–3600)
Median nadir Day	14 (range, 11–27)	13 (range, 4–23)

RESULTS

The spectrum of side effects to CHVV therapy is reported in table 1. The major toxicity was leukopenia which is detailed in table 2 for the first two cycles of chemotherapy. It should be noted that the second course of CHVV therapy was associated with an earlier occurrence of leukopenia, and in seven of 19 patients (37%) the

TABLE 3.—Results of therapy

Objective response	No. of responses among 18 patients (%)	Subjective remission	No. of remissions/ No. of patients evaluated (%)
Partial	4 (22)	Pain	9 (60)
No change	9 (50)	Bleeding	6 (86)
Progression	5 (28)	Dysuria	3 (33)
Median duration of objective response: 4.5 mos (range, 3–15)		Walking impairment	0
		Anorexia	4 (67)
		Asthenia	6 (75)
		Median duration of subjective remission: 3 mos (range, 1–15)	

Day-9 doses of vinblastine and vincristine had to be withheld due to low wbc counts.

Objective and subjective results of therapy are shown in table 3. The overall objective partial remission rate was 22% (four of 18 patients).

Our results demonstrate that the CHVV combination therapy may induce partial response in approximately the same proportion of patients as other cyclophosphamide-containing polychemotherapy regimens (4). However, at present, combination therapy including Adriamycin, *cis*-dichlorodiammineplatinum(II), and 5-fluorouracil would seem to produce better remission rates (5,6).

REFERENCES

1. PANNUTI F, ROSSI AP, PIANA E, ET AL. A new polychemotherapy schedule in the treatment of advanced stomach cancer: a pilot study. IRCS Med Sci 5:439, 1977.
2. CARTER SK, and WASSERMAN TH. The chemotherapy of urologic cancer. Cancer 36:729–747, 1975.
3. HOLLAND JF, SCHARLAU C, GAILANI S, ET AL. Vincristine treatment of advanced cancer: a cooperative study of 392 cases. Cancer Res 33:1258–1264, 1973.
4. YAGODA A, WATSON RC, GRABSTALD H, ET AL. Adriamycin and cyclophosphamide in advanced bladder cancer. Cancer Treat Rep 61:97–99, 1977.
5. CARTER SK. Chemotherapy and genitourinary oncology. I. Bladder cancer. Cancer Treat Rev 5:85–93, 1978.
6. WILLIAMS SD, ROHN RJ, DONOHUE JP, ET AL. Chemotherapy of bladder cancer with *cis*-diamminedichloroplatinum (DDP), Adriamycin (ADR), and 5-fluorouracil (5-FU). Proc Am Assoc Cancer Res and ASCO 19:316, 1978.

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On Methods for Testing and Achieving Cancer Chronotherapy[1]

The advantage of adjusting cancer treatment according to circadian rhythms, which has been documented and confirmed during the past several years (1), was questioned in a recent report by Rose et al (2). Some of the early studies (3), in addition to demonstrating an improved therapeutic index for Adriamycin in the treatment of a spontaneous breast cancer, compared two schedules of cytosine arabinoside (ara-C) treatment in mice with L1210 leukemia. Both schedules used the same total dose (240 mg/kg in each course) and frequency of administration (q3h × 8, q4d × 4), recommended on the basis of work by Skipper et al (4). On one schedule, the individual (3-hourly) dose was varied sinusoidally (S), with the highest doses given near the peak of the animals' circadian rhythm in tolerance for ara-C. On the other schedule (H, for "homeostatic"), individual doses did not vary. The endpoints, mean survival time and percent survival, were consistently and statistically significantly improved by the S schedule.

Rose et al also compared S and H schedules of ara-C administration in mice with L1210 leukemia, but they based their negative conclusions on responses to a "maximal tolerated dose" (MTD)—that which kills 10% of the animals (LD10)—rather than on a fixed total dose. Although the rationale for this approach has merits, imposing the straitjacket of an MTD on the design of such experiments seems unrealistic because of the statistical requirements for establishing a reliable LD10. Surely, using the response of single groups of ten to 20 animals to select the MTD for comparing the two treatment schedules (Rose et al) is likely to mislead. The use of healthy mice for determining an MTD that was applied to *leukemic* mice is also subject to criticism. The authors' own evidence (on page 1343), as well as our experience, suggests that leukemic mice are more tolerant of ara-C.

[1]A broader discussion of this topic is currently in press elsewhere (6).

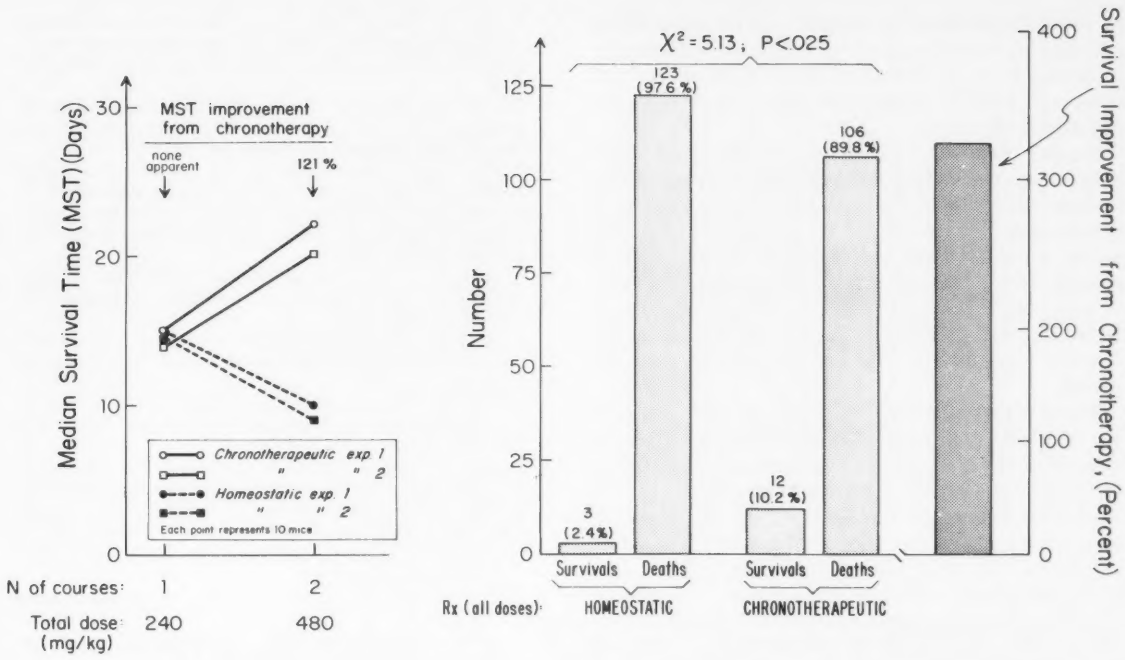

FIGURE 1.—Advantage of chronotherapeutic over homeostatic treatment of murine leukemia independently confirmed (*see* ref 2) with 2 courses (but not by single course) of ara-C and with 2 endpoints, MST (*left*) and % survival (*right*).

Presumably, when introduced into the peritoneal cavity the drug is preferentially taken up by the ascitic tumor cells. Thus, a dose selected as the MTD in control mice may be below the MTD in leukemic mice.

An added complication is that mice treated on an S schedule tolerate a larger total dose of ara-C than do those treated on an H schedule; accordingly, the MTD was different on the two schedules. The selection of such "equitoxic" MTD doses for H and S treatment of leukemic mice on the basis of the response of nonleukemic mice seems especially questionable without a consideration of dose-response relations and relative potencies.

The authors assert that doses producing > 10% mortality in nonleukemic mice, on either schedule of treatment, are "unacceptable." Yet such doses, particularly when given in two courses, considerably increased the median survival time (MST) and even resulted in some "cures" (their tables 2–4). In the case of cancer patients, one observes that doses used in the clinic are often so high that patients may die from the effects of drugs rather than from the disease itself (5). Also, one should bear in mind that patients are treated not only to prolong their lives but also with the hope that some may be cured.

The use of the MST of dying-animals-only in itself seriously biased the interpretation of results. When five of ten mice (50%) are alive at the end of a 45-day treatment period in which two S courses of ara-C are administered, with a total dose of 560 mg/kg, an MST as small as 10 days, based only on the five mice that died (their table 3), is very misleading.

Actually, the results of Rose et al in their comparable aspects confirm the earlier results (1). Thus, if one compares the groups of Rose et al (their tables 2–4) in which the same total dose was given on both the S and H schedules and for more than a single course of treatment, one sees a consistently better outcome—in terms of MST or percent increase in lifespan—on the S schedule (five of five comparisons, $P = 0.03$). Results in their tables 2 and 3 also reveal that the full advantage of the chronotherapeutic treatment schedule may not become apparent unless the treatment course is repeated. With a total dose per course of 240 mg/kg (common to both schedules), one sees that the second course on the H schedule reduces the MST while on the S schedule it increases the MST (fig 1, left).[2] With an additional two courses of treatment,

[2]Admittedly, with two courses of treatment a total dose of only 240 mg/kg on the H schedule was found to be as effective, *in terms of MST*, as was a total dose of 480 mg/kg on the S schedule. However, the purpose here is to compare schedules using the same dose; no results were shown by Rose et al for a total dose of < 400 mg/kg over two courses in the case of the S schedule, so such a comparison cannot be made.

as originally advocated by Skipper et al (4) and modified according to chronotherapeutic principles, an even greater difference in favor of chronotherapy might have been observed. Tangible evidence to support this statement is available (1).

Another view was provided by combining all of the data on survival in tables 2–4 of Rose et al, regardless of dose (fig 1, right). Such pooling may be considered questionable in view of the inconsistency of survival among different doses and schedules; nevertheless, it encompasses the various conditions examined. Although in numerical terms an increase in cures from 2.4% to 10.2% may not be impressive, it does indicate a possible fourfold improvement from the chronotherapeutic approach.

The existence of evidence favoring chronotherapy, in the very data used by Rose et al to dismiss its merits, demonstrates the importance of one's point of view in designing and interpreting experiments. In the case of ara-C treatment of murine leukemia, the advantages of chronotherapy may stem primarily from the reduced toxicity to the host. Thus, one can employ a larger total dose to kill tumor cells, with less damage to the host than is the case when the circadian tolerance rhythm is not exploited. This may explain why the benefits were apparent only after repeated courses. It may also explain why results may be less favorable when an MTD is used. However, the amelioration of host toxicity may not be the only factor contributing to the success of chronotherapy; even with doses of Adriamycin and melphalan that do not kill a single control animal, chronotherapy can make the difference between 50% and < 15% remission of murine breast cancer (7). Chronotherapy with another combination of agents, Adriamycin and *cis*-dichlorodiammineplatinum(II), has also led to the cure of a plasmacytoma (8) which had been incurable by us and, as best we know, by others.

REFERENCES

1. Halberg F, Gupta BD, Haus E, et al. Steps toward a cancer chronopolytherapy. *In* Proceedings of the 14th International Congress on Therapeutics, Montpellier, France. Paris, France, l'Expansion Scientifique Française, 1977, pp 151–196.
2. Rose WC, Trader MW, Laster WR, Jr, et al. Chronochemotherapy of L1210 leukemic mice with cytosine arabinoside or cyclophosphamide. Cancer Treat Rep 62:1337–1349, 1978.
3. Halberg F, Haus E, Cardoso SS, et al. Toward a chronotherapy of neoplasia: tolerance of treatment depends upon host rhythms. Experientia 29:909–934, 1973.
4. Skipper HE, Schabel FM, Jr, and Wilcox WS. Experimental evaluation of potential anticancer agents. XXI. Scheduling of arabinosylcytosine to take advantage of its S-phase specificity against leukemia cells. Cancer Chemother Rep 51:125–165, 1967.
5. Case Records of Massachusetts General Hospital. Weekly clinicopathological exercise. Case #2–1973. N Engl J Med 288:91–99, 1973.
6. Halberg F, Nelson W, Cornelissen G, et al. Chronochemotherapy: L1210 leukemia and beyond. Chronobiologia. In press.
7. Halberg F, Culley D, Nelson W, et al. Chronotherapy of 13762 mammary tumor in rats. Proc Am Assoc Cancer Res and ASCO 20:111, 1979.
8. Halberg F, and Delbarre F. Summary: quo vadis chronobiologia. *In* Chronopharmacology (Proceedings of the 7th International Congress of Pharmacology, Satellite Symposium on Chronopharmacology) (Reinberg A, and Halberg F, eds). Oxford, England, Pergamon Press, 1979, pp 403–426.

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